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Pretreatment with human serum butyrylcholinesterase alone prevents cardiac abnormalities, seizures, and death in Göttingen minipigs exposed to sarin vapor

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ABSTRACT

Human serum butyrylcholinesterase (Hu BChE) is a stoichiometric bioscavenger that is being developed as a prophylactic countermeasure against organophosphorus nerve agents. This study was designed to evaluate the efficacy of Hu BChE against whole-body inhalation exposure to a lethal dose of sarin (GB) vapor. Male Göttingen minipigs were subjected to: air exposure, GB vapor exposure, or pretreatment with Hu BChE followed by GB vapor exposure. Hu BChE was administered by i.m. injection 24 h prior to exposure to 4.1 mg/m^3 of GB vapor for 60 min. Electrocardiograms (ECG), electroencephalograms (EEG), and pupil size were recorded throughout exposure. Blood drawn before and throughout exposure was analyzed for blood gases, electrolytes, metabolites, acetylcholinesterase and BChE activities, and amount of GB present. Untreated animals exposed to GB vapor exhibited cardiac abnormalities and generalized seizures, ultimately succumbing to respiratory failure. Pretreatment with 3.0 or 6.5 mg/kg of Hu BChE delayed blood gas and acid–base disturbances and the onset of cardiac and neural toxic signs, but failed to increase survivability. Pretreatment with 7.5 mg/kg of Hu BChE, however, completely prevented toxic signs, with blood chemistry and ECG and EEG parameters indistinguishable from control during and after GB exposure. GB bound in plasma was 200-fold higher than plasma from pigs that did not receive Hu BChE, suggesting that Hu BChE scavenged GB in blood and prevented it from reaching other tissues. Thus, prophylaxis with Hu BChE alone not only increased survivability, but also prevented cardiac abnormalities and neural toxicity in minipigs exposed to a lethal dose of GB vapor.

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1. Introduction

Organophosphorus (OP) nerve agents such as soman (GD), sarin (GB), VX, and tabun (GA), exert their toxicity by inhibiting acetylcholinesterase (AChE) in the central nervous system (CNS) [1,2]. Exposure to OP agents results in the accumulation of acetylcholine, hyperstimulation of central and peripheral cholinergic receptors, and the resultant production and progression of toxic signs culminating with the development of generalized tonic–clonic seizures, cardiorespiratory collapse, and death [3–6]. A novel approach for counteracting OP toxicity employs the use of an enzyme bioscavenger to sequester and neutralize these compounds before they reach their physiological targets [7]. Of the enzymes tested, human serum butyrylcholinesterase (Hu BChE; EC 3.1.1.8) appears to be most appropriate for human use [8]. A dose of 200 mg of Hu BChE is envisioned as a prophylactic treatment in humans that can protect from an exposure of up to 2 × LD_{50} of GD [9]. In addition to its use as a pretreatment for a variety of wartime scenarios, it also has potential use as a pretreatment for first responders reacting to intentional/accidental nerve gas release and as a post-exposure

Abbreviations: ChE, cholinesterase; AChE, acetylcholinesterase; Hu BChE, human serum butyrylcholinesterase; OP, organophosphorus compounds; GB (sarin), O-isopropyl methylphosphonofluoridate; GD (soman), O-pinacolyl methylphosphonofluoridate; GA (tabun), O-ethyl N,N-dimethylphosphoramidocyanate; VX, O-ethyl S-2-N,N-diisopropylaminoethyl methylphosphonothiolate; CNS, central nervous system; ECG, electrocardiogram; EEG, electroencephalogram; LCt_{50}, the product of vapor concentration and exposure time that will cause death in 50% of an exposed population.

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therapy for pesticide overexposure, cocaine overdose, or succinylcholine-induced apnea [8].

For ethical reasons, the efficacy of Hu BChE cannot be investigated in humans. Therefore, the effectiveness of Hu BChE against multiple LD_{50}'s of OP nerve agents was evaluated using different animal species, including rodents and non-human primates. It was shown that pretreatment with Hu BChE alone protected mice from toxicity due to GD, GB, VX, and GA [10,11]. In addition to enhancing survivability, pretreatment with Hu BChE prevented the development of GD-induced cognitive impairments in rats [12]. Subsequent studies in rhesus monkeys showed that although a molar ratio of Hu BChE:OP of ~1.2 was required to protect against $2.1 \times LD_{50}$ of VX, a smaller ratio of 0.62 was sufficient against $3.3 \times LD_{50}$ of GD [13]. As in rats, the enzyme also protected rhesus monkeys against GD-induced behavioral deficits, which were evaluated using a spatial discrimination task. More recently, the efficacy of Hu BChE was demonstrated in guinea pigs against cumulative s.c. challenges of up to $5.5 \times LD_{50}$ of GD or $8 \times LD_{50}$ of VX [14,15]. No signs of OP poisoning were observed and all animals survived the duration of challenge. In non-human primates, four of six cynomolgus monkeys were protected against a cumulative challenge of $5.5 \times LD_{50}$ of GD. The four surviving animals did not display any immediate or delayed signs of OP toxicity as revealed by examinations of blood chemistry and hematology parameters over 14 months [16].

Most efficacy studies described to date were conducted using i.v. or s.c. challenge of OP nerve agents. The pharmacokinetics, onset time, and severity of toxic manifestations following exposure to OPs depend not only on the animal species but also on the route of entry of OP agents. Since, inhalation is the most likely route of exposure to G-type nerve agents on the battlefield or in public places, the efficacy of Hu BChE should be evaluated against a vapor challenge. The first such study was described by Allon et al. [17], who reported that exogenously administered Hu BChE was effective in protecting guinea pigs from inhalation toxicity from nose-only exposure to GD. In the present study, we investigated for the first time the efficacy of Hu BChE as a prophylactic measure against whole-body exposure to a lethal dose of GB vapor. GB is a colorless and odorless liquid at room temperature, and can be hazardous in liquid as well as vapor form due to its relatively low vapor pressure [18]. 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. Animals were pretreated with Hu BChE by i.m. injection, 24 h prior to whole-body exposure to 4.1 mg/m^3 for 60 min of GB vapor ($2.4 \times LCt_{50}$). This value is equivalent to an LCt_{99}. Pharmacokinetic studies reported previously [19] showed that Hu BChE delivered by i.m. injection attained peak activity in minipig blood at ~24 h. Electrocardiographic (ECG) and electroencephalographic (EEG) recordings and pupil size were monitored throughout exposure. Blood drawn from a surgically implanted jugular vein catheter before and throughout the exposure period was analyzed for blood gases, electrolytes, metabolites, AChE and BChE activities, and the amount of GB present in plasma and red blood cells (RBCs). Results demonstrate that prophylaxis with a dose of 7.5 mg/kg of Hu BChE alone was effective in preventing cardiac abnormalities, neural toxicity, and death due to respiratory failure in minipigs exposed to a lethal dose of GB vapor.

2. Materials and methods

All animal studies were conducted in compliance with the Animal Welfare Act and other federal statutes and regulations stated in the Guide for the Care and Use of Laboratory Animals (NRC Publication, 1996 edition). All procedures with animals received prior approval from the Edgewood Chemical and Biological Center (ECBC) Institutional Animal Care and Use Committee and were performed in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

2.1. Materials

All reagent grade chemicals including acetylthiocholine (ATC), butyrylthiocholine (BTC), 5,5-dithiobisnitrobenzoic acid (DTNB), and ethopropazine were purchased from Sigma Chemical Co. (St. Louis, MO). Hu BChE was isolated from Cohn fraction IV-4 paste [20] and stored in lyophilized form at −20 °C.

2.2. Preparation of minipigs for whole body exposure to sarin 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 [21]. The minipigs were allowed to recover for at least three days before they were used for any exposures. During that time, the catheter was flushed with heparinized saline as needed.

2.3. Procedure for the whole body exposure of minipigs to sarin vapor

O-Isopropyl methylphosphonofluoridate (sarin or GB) used in this study was obtained from the Chemical Transfer Facility, ECBC, APG, MD. Chemical agent standard analytical reagent material (CASARM)-grade GB (lot GB-U-6814-CTF-N) was verified as 98.3 ± 0.48 wt% pure as determined by quantitative ^{31}P NMR spectroscopy, and stored in sealed ampoules under nitrogen. Ampoules were used to prepare external standards or to generate GB vapor. All external standards for the quantification of GB vapor were prepared on the day of the experiment. Triethylphosphate (99.9% purity; Aldrich, Milwaukee, WI) was used as the internal standard for the analysis of purity of GB.

Whole body exposures to GB vapor were conducted by the Operational Toxicology Team, Research and Technology Directorate at ECBC, APG, MD, using a 1000-l dynamic airflow chamber. The Hazleton style chamber was constructed of stainless steel with Plexiglas doors on two sides. 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 (GC) analysis.

Each pig was placed in a canvas sling fitted to accept the animal through 4 leg holes (Lomir Biomedical, Inc., Malone, NY, or Canvas and Awning supplies, White Marsh, MD) and maintained in the sling using two straps that secured over the pig's shoulders and hips. A muzzle harness was placed over the animal's snout, and secured both laterally and ventrally to the stainless-steel framing, to prevent the animals from freely moving their head. The jugular line was exteriorized from the exposure chamber, which allowed collection of blood samples throughout the exposure period. Dermal electrodes were attached and exteriorized from the exposure chamber for ECG and EEG recordings using a Ceegraph Netlink headbox with the Sleepscan computer system (Bio-Logic Systems Corp, Mundelein, IL). Infrared images of the pupil were taken through the Plexiglas.

2.4. Efficacy of Hu BChE in 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 ($n = 2$); and (5) pretreatment with 7.5 mg/kg of Hu BChE followed by GB vapor exposure ($n = 4$). Hu BChE or saline was administered by i.m. injection, 24 h prior to whole-body exposure to GB vapor. The inhalational toxicity of the vapor form of nerve agent is described as LCt_{50}. "Ct" refers to the concentration of vapor in air (measured as mg/m^3) multiplied by time of exposure (measured in min). The concentration of GB vapor in the chamber was 4.1 mg/m^3 for 60 min, yielding an exposure of 246 mg min/m^3, which is equivalent to a 2.4 × LCt_{50}. EEG and ECG were monitored throughout exposure. Blood drawn from a surgically implanted jugular vein catheter before and at 10, 20, 30, 40, 50, and 60 min following exposure to GB vapor was analyzed for blood gases, electrolytes, metabolites, AChE and BChE activities, and the amount of GB present in RBCs and plasma.

2.5. Blood assays

Following collection, blood samples were analyzed immediately using a Portable Clinical Analyzer (i-STAT Corp., Princeton, NJ) and EG8+ cartridges. The following parameters were obtained: pH, partial pressure carbon dioxide (pCO_2), partial pressure oxygen (pO_2), Na^+, K^+, hematocrit (HCT), glucose, bicarbonate (HCO_3^-), hemoglobin (Hb), TCO_2 (total CO_2), and sO_2 (oxygen saturation).

Assays for AChE and BChE activity in whole blood were conducted using a modified Ellman assay [22]. Briefly, 10 μl of blood was lysed in 190 μl of water, and AChE activity was determined using 20 μM ethopropazine as a BChE-specific inhibitor and 1 mM ATC as the substrate [23]. BChE activity in blood was determined using 500 nM (−) huperzine A as an AChE-specific inhibitor and 1 mM BTC as the substrate. AChE and BChE activities were measured by monitoring the change in absorbance of DTNB at 412 nm for 5 min at 22 °C. One unit (U) of enzyme activity is defined as the amount that hydrolyzes one μmol of substrate in one min.

The amount of GB bound to erythrocyte and plasma fractions was determined by measuring regenerated GB using GC/mass spectrometry (GC/MS) as described [24]. Blood was separated into erythrocyte and plasma fractions by centrifugation and GB was regenerated from 0.1 to 0.25 g of each sample by adding 1 ml of acetate buffer pH 3.5 and 200 μl of 6 M KF. One microliter of 2H6-GB (50 μg/ml) was added as an internal standard; the sample was mixed, centrifuged at 1500 rpm, and the supernatant removed. The pellet was resuspended in 750 μl of acetate buffer and 200 μl of KF, vortexed, and centrifuged, and the supernatant was removed and combined with previous supernatant. The combined supernatant was loaded on a SPE C18 cartridge that was prepared by passing 1 ml of ethyl acetate, followed by 1 ml of isopropyl alcohol and 1 ml of acetate buffer. The extracted sample was eluted with 1 ml of ethyl acetate, dried, filtered through a 0.2 μm nylon Acrodisc® syringe filter (Pall Gelman Laboratory, Ann Arbor, MI), and transferred to a GC autosampler vial. The volume of the sample was reduced to 50 μl under a stream of nitrogen before being analyzed by GC/MS (Agilent Technologies, Wilmington, DE).

2.6. Analysis of ECG and EEG data

2.6.1. ECG

ECG data were converted into the IOX format and analyzed using the ECG-AUTO software (Emka Technologies, Falls Church, VA). 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. RR, PR, and QT intervals were quantified for each block.

2.6.2. 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 visually to disqualify regions of the tracing exhibiting electro-muscular contamination or other artifacts. 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 min exposure period following introduction of GB were recorded. These were fragmented into 5 min bins, and a 60 s data block from each bin was then subjected 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.

2.7. Statistical analysis

2.7.1. ECG

2.7.1.1. Correlation analysis for RR–QT intervals. To examine whether RR and QT intervals were correlated under physiological conditions, simple bivariate correlation was performed on values obtained from air-exposed control pigs, and r^2 and the F-ratio of the fit were determined.

2.7.1.2. Longitudinal analysis of cardiac parameters. Individual variance with respect to the onset of cardiac pathophysiology during GB vapor exposure (Table 1A) precluded a conventional repeated measures analysis. To address this issue, time courses for each animal that ultimately succumbed to GB vapor were aligned to 'time of death', rather than the onset of GB exposure (Table 1B). This procedure provided a more reliable and sensitive means to assess whether pretreatment with Hu BChE prevented or attenuated GB cardiotoxicity. Once aligned in this manner, the data were analyzed by means of mixed-model ANCOVA. Treatment (dose of Hu BChE), PR, RR, and QT intervals were considered between-subjects effects, while time of death and the interaction of time with each between-subjects factor were considered within-subject effects. To guard against departures from compound symmetry, univariate F-ratios were tested against Greenhouse–Geisser corrected degrees of freedom. The resulting approximate F-ratio is reported, with alpha = 0.05. Post hoc comparisons of each treatment profile with those of air-exposed control pigs were performed using the Dunnett procedure to constrain the multiple contrast error rate to an acceptable level.

2.7.2. EEG

2.7.2.1. Baseline values. The area under the curve for each of the classic EEG frequency bands was analyzed by means of a two-factor ANOVA with treatment and band as main effects. Post hoc all pairwise comparisons were performed using the Tukey-HSD procedure.

2.7.2.2. Exposure time course. EEG spectra were analyzed as a function of time using a mixed-model ANCOVA. Since baseline values were found to vary as a function of treatment group, these were used as a covariate to factor out group differences with respect to initial signal intensity. Variates were log-transformed to approximate normal distribution. As described above, degrees of

Table 1A
Un-synchronized RR intervals normalized to baseline levels.

	Blocks of Time (min)																				
Treatment	Base	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27	27-30	30-33	33-36	36-39	39-42	42-45	45-48	48-51	51-54	54-57	57-60
Air	100	2.9	1.3	-3.1	2.6	12.6	7.3	10.8	13.1	15.1	3.8	12.7	5.3	20.2	-9.0	8.8	13.6	13.7	1.6	5.0	10.4
Air	100	-9.2	-14.7	-6.5	7.2	-3.2	-6.6	-6.6	-14.3	-5.9	-14.9	5.0		-4.7	4.6	23.5	-8.9	15.6			-31.3
Air	100	-19.2	-19.9	-23.2	-18.3	-20.0	-22.3	-11.4	-15.6	-6.5	-12.4	-0.4	-5.3	-10.9	-17.2	-23.6	-22.9	-26.1	-26.5	-27.4	-22.9
Air	100	13.1	17.1	14.6	-14.4	-4.7	5.4	8.4	2.5	-2.2	5.3	-3.6	33.9	-37.4	0.1	15.7	1.9	2.0	12.2	13.0	13.8
Air	100	-5.3	0.9	-11.1	-11.4	-17.2	-13.4	-11.9	-17.7	-22.6	-9.1	-22.2	-21.6	-20.6	-23.3	-22.3	-18.7	-10.3	-2.4	-10.7	-10.9
Air	100	7.5	8.7	8.2	13.8	4.1	4.2	8.1	0.8	3.2	13.7	7.9	14.9	27.5	21.3	25.3	17.4	21.5	18.2	19.8	
Air	100	4.2	0.1	-6.8	-9.3	-7.8	-5.9	-13.7	-8.4	-4.9	-4.1	-11.7	-9.3	-8.6	-15.8	-10.2	-6.9				
Air	100	-0.9	5.0	-6.7	4.6	2.6	-4.5	0.8	4.0	0.0	4.1	9.5	-9.9	10.0		2.8	0.3			-1.7	-12.1
Air	100	-7.1	-24.5	-11.8	-14.2	-12.4	-5.1	-11.7	-13.8	-7.0	-3.4	-11.3	1.8	-7.1	-9.7	0.7					
Saline	100	-5.7	-30.5	-21.1	-36.7	-48.7	-51.4	-54.4	-47.6		25.6	76.2	618.0								
Saline	100	-14.5	-15.9	-14.5	-0.19	-18.7	-21.5	-26.6	-34.5				67.9	169.3							
Saline	100	-8.9	-8.4	-8.3	-12.2	-14.5	-21.5	-23.3	-28.3	-31.1	-39.6	-41.2	-48.6	-52.1			88.1	35.2	172.3		
Saline	100	-4.5	-1.4	-6.7	-2.11	-7.6	-12.4	-12.6	-9.9	-7.4	-5.5	-3.7	-5.8	-4.9	-4.6	-7.1	-12.3	-10.5	-11.9	-23.3	-35.2
Hu BChE-3	100	2.0	-2.5	-7.6	-18.4	-17.9	-19.8	-20.2	-18.4	-18.6	-20.7	-23.3	-28.4	-31.8	-51.4	-51.9	-31.9	6.7	73.4	312.0	
Hu BChE-3	100	-12.6	-21.1	-19.5	-15.2	-18.2	-23.8	-24.8	-28.0	-29.1					25.4	76.6	57.4				
Hu BChE-3	100	9.9	-3.5	-6.0	-2.0	-9.5	-9.7	-6.5	-4.9	-9.3	-10.6					-38.3	-42.1	-52.2			
Hu BChE-6.5	100	27.1	29.8	23.3	19.8	22.2	21.8	20.4	20.5	18.0	13.7	11.8	16.0	13.3	9.0	7.6	-13.0	-22.1			86.2
Hu BChE-6.5	100	17.9	9.7	2.7	-2.2	0.6	0.4	6.0	7.5	5.2	5.0	1.1	-6.0	-9.3	-24.6	-46.6			183.4	126.7	99.3
Hu BChE-7.5	100	-1.1	0.6	1.8	3.4	2.0	2.5	-4.3	-2.3	-3.9	-8.5	-5.8	-2.0	-1.1	-6.6	-2.3	-7.0				-1.6
Hu BChE-7.5	100	-10.7	-15.3	-8.6	-6.2	1.2	-9.2	-8.1	-8.8	-7.3	-6.3	-8.2	-4.1	-5.1	-2.9	-0.1	-2.8	-3.4	0.7	-2.5	1.3
Hu BChE-7.5	100	11.6	17.6	19.2	16.3	12.5	18.1	8.4	7.4	10.1	6.5	10.8	13.6	12.9	11.6	8.1	-7.6	15.4	5.7	8.4	0.95

Table 1B
Normalized RR intervals aligned to time of death.

	Blocks of Time (min)																				
Treatment	Base	0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27	27-30	30-33	33-36	36-39	39-41	41-44	44-47	47-50	50-53	54-57	57-60
Air	461	474	467	447	473	519	494	510	521	530	478	519	485	554	419	501	523	524	468	484	508
Air	473	419	393	431	494	446	430	430	395	434	392	484		439	482	569	420	533			316
Air	369	372	369	354	376	369	358	408	389	431	403	459	436	410	381	352	355	341	339	335	355
Air	551	521	540	528	395	439	486	500	472	450	485	444	617	288	461	533	470	470	517	521	524
Air	452	437	465	409	408	382	399	406	379	357	419	358	361	366	354	358	375	413	450	412	411
Air	475	495	501	499	524	480	480	498	464	475	524	497	529	588	559	577	541	560	545	552	
Air	472	480	461	430	418	425	433	398	422	438	442	407	418	421	388	414	429				
Air	458	457	484	430	482	473	440	464	479	461	479	504	415	507		474	462			453	405
Air	413	428	348	406	395	404	437	407	397	429	445	409	469	428	416	464					
Saline									469	435	320	364	292	237	224	211	242		579	812	3308
Saline								388	394	388	394	460	375	362	338	302				773	1241
Saline			438	420	422	423	405	394	362	353	330	318	278	271	237	221			867	623	1255
Saline	426	404	403	415	427	435	444	434	438	440	428	404	412	406	354	299					
Hu BChE-3		436	470	449	426	376	378	370	368	376	375	365	353	330	314	224	221	313	491	799	1899
Hu BChE-3					389	403	364	371	391	377	351	347	332	327					578	814	2983
Hu BChE-3	515	507	444	433	470	505	506	431	483	418	412					284	267	221			
Hu BChE-6.5	581	586	598	568	552	563	561	555	555	544	524	515	535	522	502	496	401	359			858
Hu BChE-6.5	523	543	505	473	451	464	463	489	495	485	484	456	433	418	347	246			1306	1045	918
Hu BChE-7.5	443	456	463	469	477	470	472	441	450	443	422	434	452	456	531	450	429				454
Hu BChE-7.5	428	411	390	421	432	466	418	423	420	427	432	423	442	437	447	460	448	445	464	449	467
Hu BChE-7.5	529	514	542	549	536	518	544	499	494	507	490	510	523	520	513	498	496	534	487	499	465

Color codes indicate the following: RR intervals that were maintained within 20% of the baseline value are coded in green as 'normal'; RR intervals shorter than 20% are coded in yellow as 'tachycardic'; RR-intervals >20% are coded in red as 'bradycardic'. Data gaps in the trace (typically due to noise) where less than 20 valid beats/3 min bin were observed are coded purple. Cardiac death is indicated in black.

freedom were adjusted using the Greenhouse–Geisser procedure. Treatment (dose of Hu BChE), frequency band and higher order interactions were considered between-subjects effects, while time of death and the interaction of time with each between-subjects factor were considered within-subject effects. As mentioned above, the alignment of time courses for each animal that ultimately succumbed to GB vapor to 'time of death' provided a more reliable and sensitive means to assess whether pretreatment with Hu BChE prevented or attenuated GB neurotoxicity. Post hoc comparisons of each treatment profile with those of air-exposed control pigs were performed using the Dunnett procedure to constrain the multiple contrast error rate to an acceptable level.

3. Results

3.1. Circulating cholinesterase activity following exposure of minipigs to sarin vapor

Minipigs were pretreated with saline, 3.0, or 7.5 mg/kg of Hu BChE by i.m. injection and challenged with air or GB vapor 18–20 h

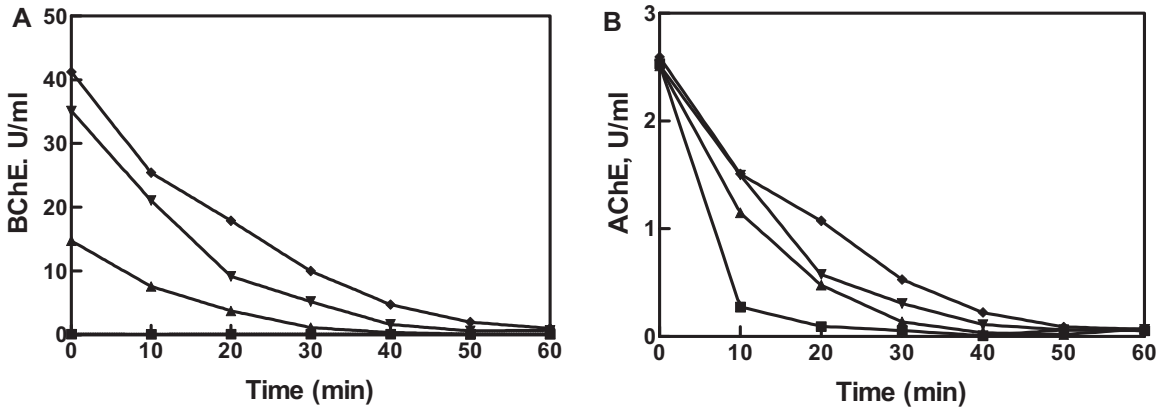

Fig. 1. Circulating BChE (panel A) and AChE (panel B) levels in the blood of minipigs injected with saline or Hu BChE and exposed to GB vapor. All values are mean ± S.E.M. The symbols represent the following pretreatments prior to exposure to GB vapor: (■), saline; (▲), 3 mg/kg of Hu BChE; (▼), 6.5 mg/kg of Hu BChE; (◆), 7.5 mg/kg of Hu BChE.

later. However, due to an error in preparing the enzyme solution, two animals received 6.5 mg/kg of Hu BChE instead of 7.5 mg/kg. The average baseline AChE and BChE activities in the blood of all animals were 2.54 ± 0.06 and 0.16 ± 0.03 U/ml, respectively. As expected, circulating blood BChE activity reached 14.8 ± 2.1, 35.1 ± 1.9, and 41.2 ± 0.9 U/ml at 18–20 h following the administration of 3.0, 6.5, or 7.5 mg/kg of Hu BChE by i.m. injection (Fig. 1A). The administrations of these doses of Hu BChE did not affect circulating AChE activity. When saline-treated animals were exposed to GB vapor (4.1 mg/m^3), circulating AChE activity decreased to ~10% of baseline value after 10 min into exposure and was completely inhibited by 20 min (Fig. 1B). On the other hand, in animals that were pretreated with 3.0, 6.5, or 7.5 mg/kg of Hu BChE, the exogenously administered enzyme neutralized agent in circulation (Fig. 1A) and delayed the inhibition of circulating AChE activity by GB in a dose-dependent manner (Fig. 1B). As shown in Fig. 1, pretreatment with 3.0 or 6.5 mg/kg of Hu BChE did not provide sufficient enzyme to bind all agent in circulation, and therefore was ineffective in protecting minipigs against an exposure to 4.1 mg/m^3 of GB vapor for 60 min. Animals started to display cholinergic symptoms when AChE and BChE activities in blood were neutralized by GB, and ultimately died due to respiratory failure. However, a dose of 7.5 mg/kg of Hu BChE was sufficient for sequestering most of the GB in circulation and preventing toxicity due to GB vapor. This was also substantiated 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 or from the last blood sample collected prior to death. An increase in the amount of GB in plasma that correlated with the dose of Hu BChE was observed, and was about 200-fold greater in pigs pretreated with 7.5 mg/kg of Hu BChE compared to control animals (Fig. 2). However, the amount of GB detected in RBCs was similar in control and all Hu BChE pretreated pigs.

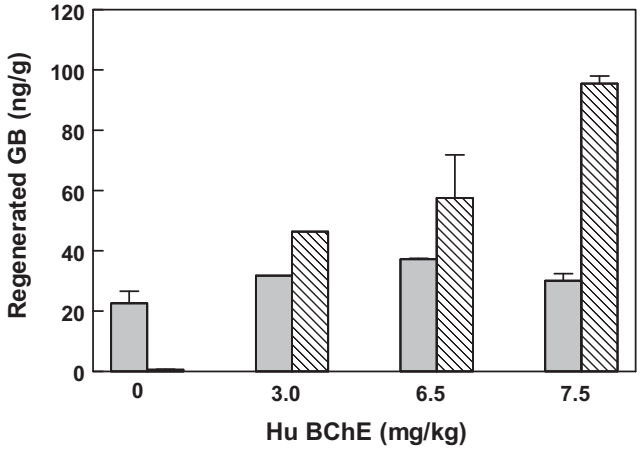

Fig. 2. Regenerated GB in RBCs and plasma from saline and Hu BChE pretreated minipigs following exposure to GB vapor or air. Values are shown for venous blood taken from animals pretreated with saline or Hu BChE (3.0–7.5 mg/kg) at 60 min post-GB or air exposure, or from the last sample collected before death of the animal. All values are mean ± S.E.M. Blood was separated into erythrocyte (solid bars) and plasma (hatched bars) fractions by centrifugation and GB was regenerated from 0.1 to 0.25 g of each sample as described [24].

3.2. Effect of Hu BChE pretreatment on GB vapor-induced changes in blood chemistry parameters

Baseline and terminal blood chemistry parameters in minipigs that were exposed to GB vapor following pretreatment with saline (untreated) or various doses of Hu BChE are shown in Table 2. Minipigs that received saline or pretreatment with 3.0 or 6.5 mg/kg of Hu BChE exhibited terminal values consistent with an acute respiratory failure characterized by hypoxemia, hypercapnia, acute respiratory acidosis, and low blood sO_2. In untreated animals, blood pO_2 levels started to decline within minutes following GB exposure, and a sharp increase in blood pCO_2, a decrease in blood HCO_3^-, and acidosis became apparent at 20 min into GB exposure (data not shown). Corresponding changes in terminal blood pCO_2 and HCO_3^- indicated mixed metabolic acidosis and respiratory acidosis, which were caused by respiratory failure. An increase in Hb, which is used for buffering carbonic acid to produce HCO_3^-, was also observed. Metabolic acidosis caused the movement of intra-cellular K^+ into the extra-cellular compartment to compensate for excess hydrogen ions, which resulted in overt hyperkalemia. Serum glucose levels also increased following the 60 minute GB exposure. Compared to the untreated group, animals pretreated with 3.0 or 6.5 mg/kg Hu BChE presented delayed blood gas and acid–base disturbances following exposure to GB vapor, but they eventually died due to respiratory failure. However, the blood chemistry parameters of GB-exposed pigs pretreated with 7.5 mg/kg Hu BChE were similar to those of air-exposed pigs, suggesting that the pretreatment prevented respiratory failure.

3.3. Establishment of baseline ECG and EEG parameters in control minipigs

ECG data from air-exposed control minipigs were used to establish normal baseline data for cardiac heart rate, rhythmicity, RR, PR, and QT intervals. A comparison of these values with

Table 2
Effect of Hu BChE pretreatment on key blood chemistry parameters in minipigs exposed to GB vapor.

Parameter	Air		Saline + GB		3.0 mg/kg Hu BChE + GB		6.5 mg/kg Hu BChE + GB		7.5 mg/kg Hu BChE + GB	
	Baseline	Terminal	Baseline	Terminal	Baseline	Terminal	Baseline	Terminal	Baseline	Terminal
Glucose (mg/dl)	101 ± 7	106 ± 5	100 ± 4	181 ± 9$^{**,\#\#}$	102 ± 6	171 ± 0$^{**,\#\#}$	108 ± 1	288 ± 0$^{*,\#\#}$	105 ± 5	126 ± 11
Na^+ (mmol/l)	140 ± 1	139 ± 1	138 ± 1	144 ± 2	145 ± 2	146 ± 3	141 ± 1	144 ± 1	139 ± 0	140 ± 1
K^+ (mmol/l)	4.3 ± 0.3	4.2 ± 0.1	4.1 ± 0.1	7.5 ± 0.4$^{**,\#\#}$	4.1 ± 0.1	5.6 ± 0.1$^{**,\#\#}$	4.0 ± 0.2	5.2 ± 0.1$^{\#\#}$	4.2 ± 0.1	4.4 ± 0.3
HCT (%)	34.0 ± 1.0	30.7 ± 1.0	31.5 ± 1.5	38.5 ± 0.5$^{\#\#}$	35.3 ± 1.5	40.5 ± 0.5$^{\#\#}$	29.0 ± 5.0	38.5 ± 2.5$^{\#\#}$	29.3 ± 3.7	30.3 ± 3.7
Hb (mg/dl)	11.1 ± 0.3	10.4 ± 0.3	10.7 ± 0.5	13.0 ± 0.1$^{\#\#}$	11.9 ± 0.5	13.8 ± 0.1$^{\#\#}$	9.9 ± 1.7	13.1 ± 0.8	10.0 ± 1.2	10.3 ± 1.3
pH	7.38 ± 0.03	7.42 ± 0.01	7.42 ± 0.05	6.67 ± 0.00$^{**,\#\#}$	7.36 ± 0.02	6.85 ± 0.01$^{**,\#\#}$	7.46 ± 0.01	6.88 ± 0.02$^{**,\#\#}$	7.38 ± 0.03	7.42 ± 0.03
pCO_2 (mmHg)	48.6 ± 1.1	49.7 ± 1.5	38.5 ± 4.8	119.4 ± 6.7$^{**,\#\#}$	47.3 ± 2.3	84.0 ± 1.0$^{**,\#\#}$	46.7 ± 1.9	101.1 ± 5.7$^{**,\#}$	49.4 ± 3.2	46.9 ± 1.2
pO_2 (mmHg)	37.7 ± 1.9	37.4 ± 1.2	45.3 ± 3.5	24.7 ± 2.0$^{**,\#\#}$	44.0 ± 3.8	24.5 ± 2.5$^{**,\#}$	44.5 ± 4.5	5.0 ± 0.0$^{**,\#\#}$	40.0 ± 3.0	35.7 ± 2.2
TCO_2 (mmol/l)	32.5 ± 1.3	34.0 ± 1.3	29.7 ± 0.7	21.3 ± 2.2$^{**,\#\#}$	30.3 ± 0.9	16.5 ± 1.5$^{**,\#\#}$	27.3 ± 6.8	22.0 ± 2.0	30.3 ± 1.8	32.0 ± 1.5
HCO_3^- (mmol/l)	30.9 ± 1.4	32.5 ± 0.9	28.3 ± 0.8	18.2 ± 2.6$^{**,\#\#}$	28.0 ± 0.6	14.2 ± 1.2$^{**,\#\#}$	26.3 ± 6.3	18.9 ± 1.9$^{\#}$	29.1 ± 1.7	30.8 ± 1.7
sO_2 (%)	74.8 ± 1.3	71.0 ± 1.3	81.0 ± 2.6	25.0 ± 6.1$^{**,\#\#}$	77.3 ± 3.7	18.5 ± 4.5$^{**,\#\#}$	84.5 ± 1.5	24.0 ± 12.0	72.3 ± 4.4	68.7 ± 4.3

All values are mean ± S.E.M. Baseline values were measured from venous blood taken before air or GB vapor exposure commenced and terminal values were measured from venous blood taken at 60 min post-GB or air exposure, or from the last sample collected before death of the animal.
Abbreviations are described in Section 2.
* $p < 0.05$ *vs* baseline values.
** $p < 0.01$ *vs* baseline values.
$^{\#}$ $p < 0.05$ *vs* air control group.
$^{\#\#}$ $p < 0.01$ *vs* air control group.

previously published data is presented in Table 3. The mean RR and QT intervals observed in this study were somewhat shorter than those published for similarly aged pigs, but were within the 95% confidence limits of referenced values. A weak but significant correlation was observed for RR and QT intervals [$r^2 = 0.50$, $F_{(1,7)} = 7.08$, $p < 0.05$]. PR intervals for air-exposed pigs were similar to reference values.

A typical EEG power spectrum obtained from an air-exposed pig demonstrated low amplitude (peak <300 μV) and long wavelength energy predominantly within the delta and theta frequency bands. Although we could not find any previously published spectral analyses of minipig EEG tracings, the spectrum we obtained is consistent with that published for the rhesus monkey [25]. EEG power spectra of control pigs were stable throughout the period of air exposure.

3.4. Baseline ECG and EEG parameters in minipigs following pretreatment with Hu BChE

A comparison of ECG parameters from groups of animals that received pretreatment with different doses of Hu BChE revealed that enzyme pretreatment tended to result in a reduced baseline HR, although the main effect of dose was not statistically significant [$F_{(4,23)} = 2.85$, $p = 0.05$].

Differences in baseline EEG power were observed as a result of Hu BChE pretreatment ($F_{(4,80)} = 8.20$, $p < 0.0001$), and all pairwise means comparisons revealed a dose-dependent effect; total EEG power increased as the dose of Hu BChE was increased. Since both air-exposed control and untreated groups received identical treatments (saline) prior to baseline, a post hoc contrast was performed between saline- and Hu BChE-pretreated groups. No statistically significant differences between the two groups were revealed with respect to EEG power ($F_{(1,80)} = 0.05$, $p = 0.82$), suggesting that this effect in the Hu BChE-pretreated group reflects artifactual differences in basal signal intensity.

3.5. Effect of Hu BChE pretreatment on GB vapor-induced cardiac abnormalities

Whole body exposure to GB vapor resulted in the development of a variety of cardiac abnormalities in the minipigs. However, the onset of toxicity as a result of GB vapor exposure was highly variable between subject groups, as exemplified by RR intervals. In a previous study it was reported that the normalization of chronology of events using 'time of death' made the appearance of gross clinical signs more consistent [26]. Therefore, to allow for statistical analysis of the effect of different doses of Hu BChE on cardiac parameters, it was necessary to synchronize the timelines to 'time of death'. Tachycardia, with corresponding shortening of RR, PR and QT intervals, occurred in all pigs that ultimately succumbed to GB intoxication (Fig. 3). While rate disturbances were generalized features of GB intoxication, tachycardia typically occurred in sinus rhythm, and arrhythmias were rarely observed prior to the onset of seizures. Status epilepticus was generally followed by profound sinus bradycardia. Desynchronization of atrial and ventricular rates was often observed, usually with premature junctional or ventricular complexes, prior to complete cardiac arrest. Interestingly, PR lengthening, while evident, was nevertheless a relatively subtle feature of the post-seizure, terminal phase of cardiac function, and AV conduction abnormalities per se appeared rather uncommon. A regular but progressively slowing junctional or ventricular rhythm was observed immediately prior to complete cardiac arrest (Fig. 3). Neither QT

Table 3
Comparison of previously published ECG parameters[a] for minipigs with those obtained in the present study.

Species	Age (mo)	Weight (kg)	Sex	*n*	HR (bpm)	RR (ms)	PR (ms)	QT (ms)	Reference
n/r	7	~18	M/F	7	123 (110–160)	n/r	117 (100–130)	250 (230–280)	Dukes and Szabuniewicz [49]
Göttingen	n/r	~12	M/F	204	111 (64–190)	n/r	88 (63–120)	252 (183–353)	Eckenfels and Schuler [48]
Göttingen	5	~9	M/F	12	103 (91–121)	527 (498–630)	84 (70–96)	290 (256–320)	Nahas et al. [47]
Göttingen	15	~30	F	15	104 (95–112)	n/r	122 (112–132)	360 (335–385)	Schuleri et al. [46]
Göttingen	4–5	~8	M	9	134 ± 23	458 ± 49	89.8 ± 11	234 ± 16	This study

n/r; not reported.
[a] Cardiac electrophysiological parameters for minipigs were obtained via standard limb lead II electrocardiography. Uncorrected QT intervals are presented for ease of comparison.

prolongation nor Torsades des Pointes or other classical ventricular arrhythmias were noted.

Pretreatment with Hu BChE dose-dependently delayed the onset of cardiac abnormalities, with 7.5 mg/kg preventing the manifestation of rate abnormalities, seizures, and death altogether (Table 4). Similar to untreated animals, a shortening of PR, RR and QT intervals was observed in all animals pretreated with 3.0 mg/kg Hu BChE, except that these effects were delayed (Fig. 3). When the dose of Hu BChE was increased to 6.5 mg/kg, only mild shortenings in RR, PR and QT intervals were observed (data not shown). However, the profiles of air-exposed and GB-exposed pigs pretreated with 7.5 mg/kg Hu BChE were indistinguishable, suggesting that the pretreatment provided substantial cardiac protection (Fig. 3).

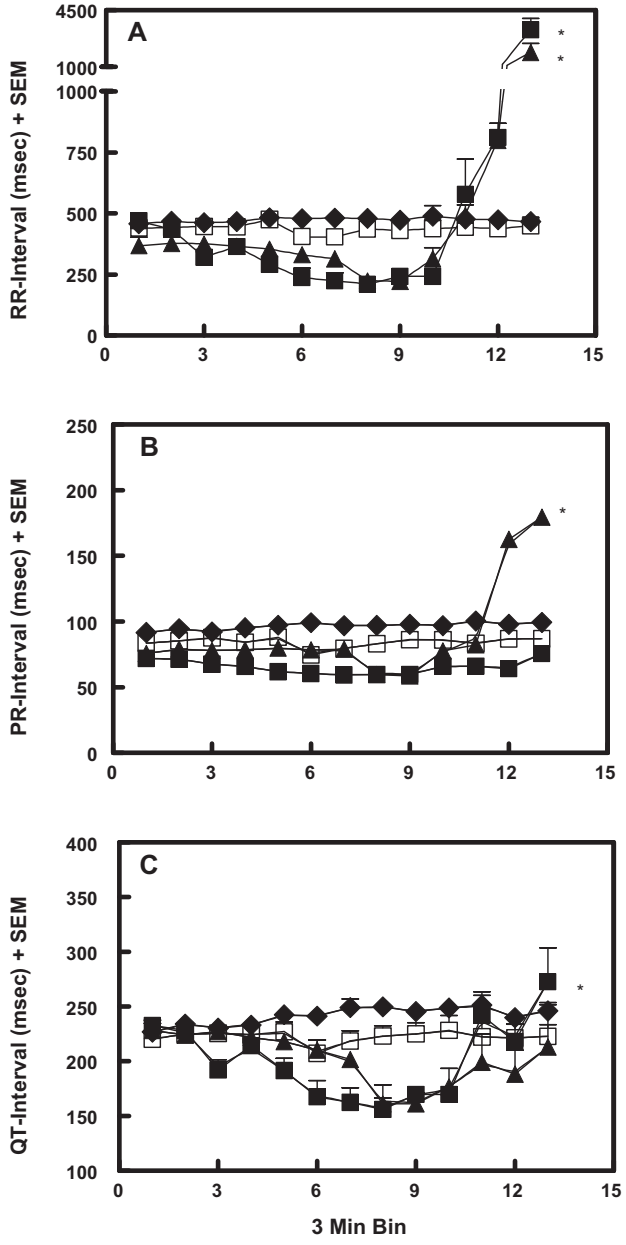

Fig. 3. RR, PR, and QT intervals for minipigs exposed to GB vapor with or without pretreatment with Hu BChE. Göttingen minipigs were treated with saline or Hu BChE 24 h prior to exposure to air or 4.0 mg/m^3 GB vapor for 60 min or until death occurred. RR (A), PR (B), and QT (C) intervals were analyzed as a function of time relative to death. The symbols represent the following pretreatments prior to exposure to GB vapor: (■), saline; (▲), 3 mg/kg of Hu BChE; (◆), 7.5 mg/kg of Hu BChE, while (□) represents exposure to air. *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 two minipigs that received 6.5 mg/kg of Hu BChE; this data was therefore not included in the analysis.

Table 4
Mean time for the onset of toxic signs and death as a result of GB exposure.

Treatment	Mean time for onset of toxic signs (min)			
	Tachycardia	Seizure	Bradycardia	Death
AIR	–	–	–	–
SALINE	9.0 ± 6.0	33.0 ± 10.7	39.8 ± 8.3	45.5 ± 7.9
Hu BChE (3.0 mg/kg)	14.0 ± 4.6	37.0 ± 14.8	45.0 ± 5.2	52.0 ± 4.6
Hu BChE (6.5 mg/kg)	28.5 ± 2.1	48.0 ± 4.2	52.5 ± 6.4	60.0 ± 0.0
Hu BChE (7.5 mg/kg)	–	–	–	–

3.6. Effect of Hu BChE pretreatment on GB vapor-induced neurotoxicity

As expected, whole-body exposure to GB vapor resulted in the development of generalized tonic–clonic seizures in minipigs. Minipigs that received saline in lieu of Hu BChE exhibited a sharp increase in total power (peak amplitude of 500 μV) with the onset of generalized seizures 30–40 min into GB exposure (Fig. 4B). Seizure onset was associated with redistribution of the power spectra away from delta and into higher frequency bands (Fig. 4B). Interictal periods were rarely observed, and spike/wave activity rapidly progressed to status epilepticus and brain death, generally within 10 min after epileptogenesis. Animals that were pretreated with 3.0 and 6.5 mg/kg Hu BChE also developed generalized seizures. However, mean latency to onset appeared to be dose-dependently delayed (Fig. 4C and D). Statistical analysis confirmed a highly significant interaction between time and Hu BChE dose ($F_{(33,539)} = 6.55$, $p < 0.0001$), suggesting that bioscavenger pretreatment indeed altered EEG spectral profiles (power spectrum as a function of time). Interestingly, peak amplitude was unaffected by Hu BChE pretreatment and was in fact highest in animals pretreated with 6.5 mg/kg Hu BChE (Fig. 4D). Notably, administration of 7.5 mg/kg Hu BChE completely prevented seizure activity (Fig. 4E). Analysis of spectral frequency partitioning revealed increased distribution of EEG energy into alpha and beta bands in these animals pretreated, but total EEG power remained comparable to air-exposed controls throughout GB exposure. Pretreatment with 7.5 mg/kg Hu BChE appeared to completely prevent neural intoxication, with EEG parameters indistinguishable from control both during and 1 week after GB exposure (Fig. 4F).

4. Discussion

Due to the inability of current antidotal regimens to provide complete protection against OP nerve agent toxicity, several alternatives such as pyridostigmine bromide and enzyme bioscavengers are being considered as prophylactic countermeasures [7]. Of the enzymes evaluated, Hu BChE appears to be most suitable for human use and is currently under advanced development. Hu BChE has been previously shown to be safe, to exhibit favorable pharmacokinetics, and to effectively protect rodents, guinea pigs, and monkeys from OP intoxication after parenteral, i.v., s.c. and intranasal inhalation exposure [27]. Since G-type nerve agents are likely to be encountered as vapors and the manifestation of toxic symptoms depends on the agent and the route of exposure [28,29], this study was undertaken to assess the efficacy of Hu BChE pretreatment against whole-body exposure of Göttingen minipigs to a lethal dose (LCt_{99}) of GB vapor. As shown in Table 2, all untreated animals developed severe respiratory stress characterized by hypoxemia, hypercapnia, and acute respiratory acidosis and ultimately died due to respiratory failure. In these animals, nearly 2-fold increases in glucose and K^+ were also observed. In a

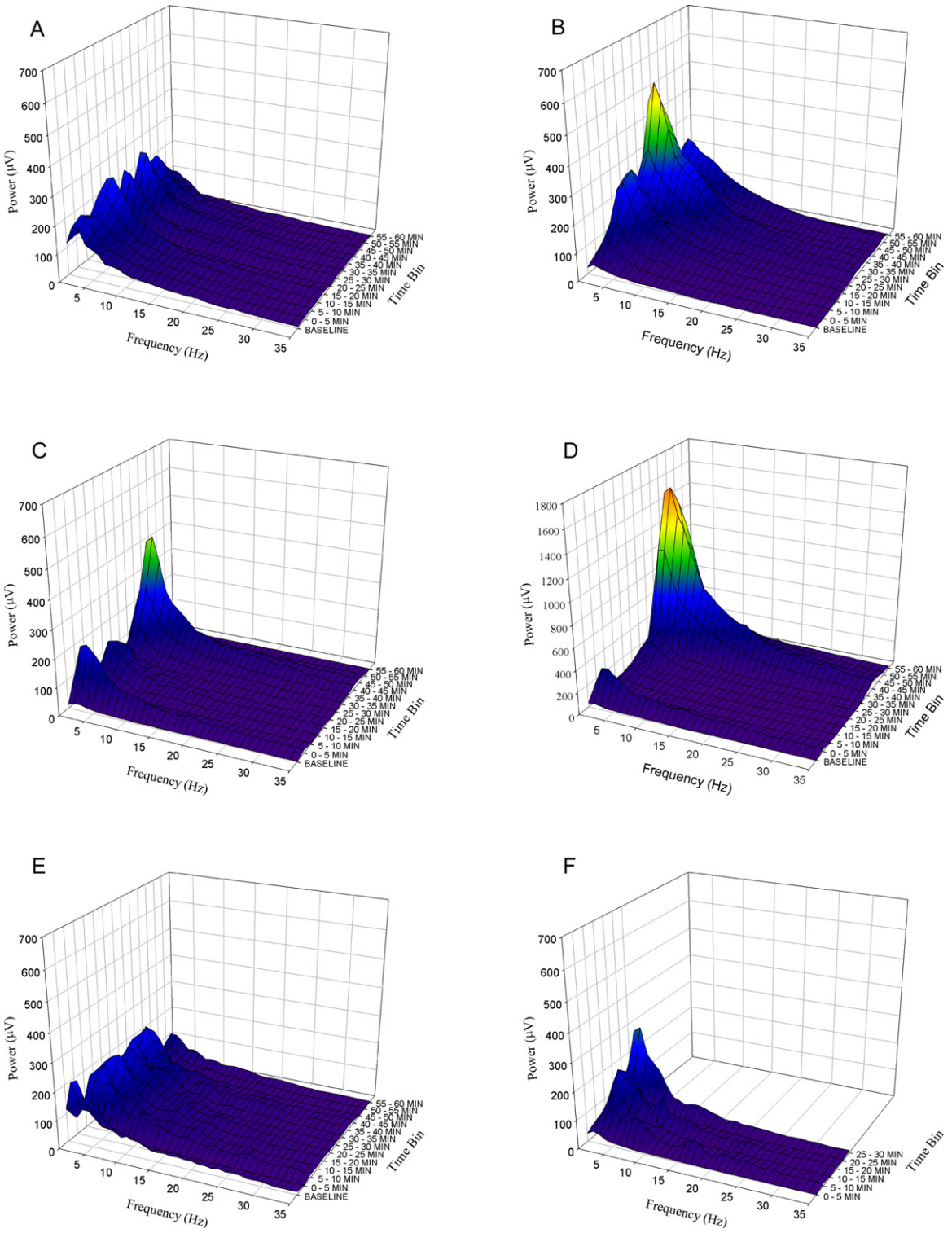

Fig. 4. 3D plots of EEG power spectra as a function of time from onset of GB vapor exposure. (A) Saline-injected, air-exposed control minipigs featured stable low amplitude, long wavelength energies. (B) Untreated minipigs exposed to GB exhibited marked increases in EEG power concurrent with onset of 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. (E) Pretreatment with 7.5 mg/kg Hu BChE prevented the manifestation of high amplitude EEG energy, and baseline spectra were similar to saline-injected animals. (F) Spectra were also normal 1 week after pretreatment with Hu BChE and GB exposure.

previous study, hyperglycemia was also reported in domestic pigs following percutaneous exposure to GD [30]. No changes in glucose utilization were observed following the percutaneous exposure of pigs to VX [26]. However, hyperkalemia was reported in pigs that were exposed to GD or VX. Thus, regardless of the route of exposure, GB is similar to GD in that it causes an increase in glucose utilization in the pig and is similar to GD and VX in that it causes an increase in K^+.

The first efficacy evaluation was conducted using a dose of 3 mg/kg of enzyme, which was projected to protect humans from

an exposure to 2 × LD_{50} of GD [9]. Although none of the animals pretreated with this dose of enzyme survived the 60 min exposure, these animals showed a delayed occurrence of respiratory stress, acid–base disturbances, sinus tachycardia, and seizures as compared to untreated animals. Subsequently, the dose of Hu BChE was increased to 7.5 mg/kg, and all animals pretreated with this dose survived the 60 min exposure to 4.1 mg/m^3 of GB vapor. In one experiment, the dose was reduced to 6.5 mg/kg, but all animals in this group eventually died due to respiratory failure, suggesting that a dose of 7.5 mg/kg of Hu BChE was necessary for the animals to survive the lethal exposure to GB vapor. However, pretreatment with even sub-optimal doses of Hu BChE, while failing to ultimately protect against the lethality of GB vapor, nevertheless delayed intoxication (Table 4). Analysis of circulating AChE and BChE activities demonstrated that pretreatment with Hu BChE protected circulating AChE from inhibition by GB in a dose-dependent manner (Fig. 1). These results, together with those of a fluoride ion-based regeneration assay (Fig. 2), confirm the proposed function of Hu BChE as a bioscavenger—that of sequestering GB in circulation.

To determine if pretreatment with Hu BChE also prevented acute cardiac abnormalities and neurological toxicity observed in minipigs exposed to GB vapor, ECG and EEG spectra recorded throughout exposure were analyzed. Untreated, GB-exposed pigs exhibited a variety of cardiac and neural abnormalities resulting from the accumulation of acetylcholine and overstimulation of cholinergic synapses at central as well as peripheral nerve endings. Classically, the clinical effects of OP intoxication on the cardiovascular system may be described in three phases [31]. At first, a transient phase of sinus tachycardia and hypertension are dominant, which is believed to be caused by intense sympathetic tone. This is followed by a prolonged second phase of extreme parasympathetic tone causing sinus bradycardia, hypotension, abnormal atrio-ventricular (A-V) conduction, and changes in S-T segment, which usually begins within minutes after intoxication and may last several hours. In the third phase, one may observe QT prolongation, polymorphic ventricular tachycardia or Torsade de points, and sudden cardiac death [31–33]. In the present study, evidence of cardiac abnormalities in minipigs exposed to GB vapor included significant and progressive sinus tachycardia followed by sinus bradycardia, abnormalities in atrial depolarization including complete atrial arrest, bradycardia of non-sinus origin, and ultimately complete cardiac arrest. Similar observations were also made in baboons exposed to various doses of GD by i.v. infusion. Most animals showed sinus tachycardia followed by sinus bradycardia that progressed to second- and third-degree A-V block [34]. All animals that were exposed to 2 × LD_{50} of GD showed a decrease in heart rate of more than 50% from baseline during the 10 min period of infusion. Rats exposed to GD or GB by a bolus injection or to GB vapor showed QT segment prolongation [35,36]. QT prolongation, ventricular tachycardia, and fibrillation were reportedly observed immediately prior to cardiac death [35]. QT prolongation is commonly reported in survivors of OP pesticide poisoning [37,38].

The actions of nerve agents and OP pesticides on the cardiovascular system can be due to direct toxic effects on the heart and/or central actions of the nerve agent. Both sympathetic and parasympathetic over-activity have been shown to cause myocardial damage [39]. Nerve agents can also cause tachycardia via stimulation of the adrenal medulla. They appear to bind to nicotinic, cardiac muscarinic, and glutamate *N*-methyl-D-aspartate (NMDA) receptors directly, suggesting that they may have additional mechanisms of action yet to be defined [40–42]. In our study, all animals that exhibited cardiac rhythm disturbance also displayed severe respiratory stress. The resultant hypoxemia, acidosis, or electrolyte derangements can act as triggers for initiating cardiac arrhythmias. These factors can also cause direct toxic effects on the cardiovascular system such as depression of myocardial contractility, sympathetic over-activity, and resistance to the effects of catecholamines, which can produce arrhythmias and even cardiac arrest [43].

Neural signs included the precipitous development of generalized seizures characterized by marked increases in total EEG power and redistribution of EEG energy into high-frequency alpha and beta bands, culminating rapidly in brain death. Focal spike and wave discharges were likely to be initiated in particularly sensitive brain regions, for example the limbic telencephalon, and to generalize through recruitment of excitatory amino acid neurotransmission before rapidly progressing to sustained convulsive seizures or status epilepticus [44]. In the rat, subcutaneous exposure to GD produced alterations in EEG coincident with the development of convulsions. These were characterized by an increase in EEG amplitude from 300 to >900 μV, with an increase evident in all spectral bands [45].

In our study, ECG spectra for air-exposed minipigs were used for establishing normal baseline values. The values for cardiac heart rate, rhythmicity, RR, PR, and QT intervals were within the 95% confidence limits of reported values for pigs of similar age [46–49]. Similarly, the EEG power spectrum obtained from an air-exposed pig was consistent with that published for the rhesus monkey [25]. EEG power spectra of control pigs were stable throughout the period of air exposure. Baseline ECG and EEG parameters in Hu BChE-treated minipigs were similar to those of saline-treated controls, suggesting that pretreatment with Hu BChE alone did not substantially alter any of the endpoints followed in the study. Animals pretreated with sub-optimal doses of Hu BChE prior to exposure to GB vapor displayed cardiac profiles that were very similar to those of untreated animals. These animals also developed generalized seizures, just like untreated control animals. The onset of symptoms was delayed in a dose-dependent fashion, but these animals died from hypoxia and/or seizures. Remarkably, pretreatment with 7.5 mg/kg Hu BChE appeared to completely prevent cardiac abnormalities and neural intoxication, with ECG and EEG parameters indistinguishable from control both during, and 1 week after, GB exposure. These results clearly demonstrate that Hu BChE conferred protection by sequestering GB in circulation. When sub-optimal doses of Hu BChE were administered, GB that was not captured by Hu BChE in blood entered the CNS to exert its toxic effects. Since Hu BChE is a 340 kDa molecule, it cannot cross the blood–brain barrier to sequester GB in the CNS. Taken together, these results demonstrate that prophylaxis with a dose of 7.5 mg/kg of Hu BChE alone was not only effective in increasing survivability, but also prevented cardiac abnormalities and neural toxicity in minipigs exposed to a lethal dose of GB vapor.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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