Case Study of a Breacher: Investigation of Neurotrauma Biomarker Levels, Self-reported Symptoms, and Functional MRI Analysis Before and After Exposure to Measured Low-Level Blast

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ABSTRACT We report a case study on a single military member who received moderate blast overpressure (OP) exposure during routine breacher training. We extend previous research on blast exposure during training, which lacked sufficient data to assess symptom profiles and OP exposure. The present work was conducted because a subjective symptom profile similar to that seen in sports concussion has been reported by military personnel exposed to blast. Data collection for this study was carried out under a research protocol approved by the relevant Human Subjects Review Committees on one subject, who received the highest OP exposure during training. The volunteer was a 20-year-old male with no prior history of traumatic brain injury (TBI) or blast exposure. The volunteer was part of a breacher training team that completed a 2-week explosive entry course. The course included 3 classroom days and 9 days of practical training, held in the morning, afternoon, and evening sessions. Blast exposure occurred on five of the nine practical training days, with multiple exposures over the course of each day. Assessments of serum, self-reported symptoms, magnetic resonance imaging, and blast characterization were conducted. Results indicated changes in glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 postblast exposure but did not manifest changes in spectrin-derived breakdown product 150 or magnetic resonance imaging. No additional symptoms were reported by the subject. Objective markers of mild TBI remain elusive, but support for serum biomarkers as an early detection mechanism is promising. Additionally, this case study demonstrated an association between OP and high level of neurotrauma biomarker in an individual.

INTRODUCTION

This manuscript reports a case study on a single 20-year-old male soldier who received a moderate blast overpressure (OP) exposure during routine training involving explosive breaching and had subsequent sleep disturbance and blood-based biomarker perturbation commonly associated with traumatic brain injury (TBI). This work extends a previous report on a pilot study of blast exposure during training, but which lacked sufficient data to assess symptom profiles and OP exposure for the entire sample. The present work was conducted because a subjective symptom profile similar to that seen in sports concussion has been reported by military personnel exposed to blast.[1] Blast exposure is reported as a subclinical brain perturbation.[2] Diagnosing this perturbation, or mild traumatic brain injury (mTBI) in general, remains a clinical challenge. Additionally, the blast does not always manifest symptomology across personnel with similar exposure profiles.[2] Therefore, this work extends the existing literature by characterizing and furthering understanding between blast OP and health outcomes.

Explosive breaching requires the use of controlled detonation to force entry into a structure. Safe standoff distance (SSD) is calculated for a given explosive weight to ensure that safe levels of estimated blast OP (typically determined as a peak incident pressure of greater than 4.0 psi [28 kPa]) are not exceeded for exposed personnel. Notably, peak pressure safety standards do not account for the total impulse pressure or the duration of the OP pulse experienced. This point will be revisited in detail later in this manuscript.

Converging evidence[2] suggests that there are elevations in biomarker loading, cognitive impairments, and increases in symptomology resulting from exposure to low-level blast (LLB) among some individuals. The SSD is an empirical algorithmic extrapolation from free-field explosions and does not translate well to dynamic urban breaching environments. Thus, quantitative measures of pressure need to be evaluated against individual outcome measures.[2] The present study augments the previous pilot study by evaluating daily, individualized pressure exposure data in addition to the data streams reported in the pilot study.[2]

Several brain-enriched or central nervous system-specific biomarkers have been shown to provide diagnostic and/or prognostic information about TBI (from severe to mild).[3,4] Such markers include neuronal-cell body-protein ubiquitin C-terminal hydrolase-L1 (UCH-L1), axonally enriched cytoskeletal protein alphaII-spectrin-derived breakdown products (SBDPs), phospho-neurofilament protein-heavy, glial fibrillary acidic protein (GFAP), and its breakdown

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product (GFAP-BDP). Both UCH-L1 and GFAP/GFAP-BDP have the ability to detect mild-to-moderate TBI.[3,4] In addition, two recent studies independently confirmed the utilities of GFAP-BDP and its combination with UCH-L1 as diagnostic and/or prognostic tool for TBI.[5,6] Although Tate et al.[2] measured three candidate brain biomarkers that might be responsive to blast-induced brain perturbation (SBDP150, UCH-L1, GFAP), GFAP will be the main biomarker of this case study. GFAP has been demonstrated to correlate more strongly with focal mass injuries than diffuse axonal injury.[7] Validation studies have shown no elevation in GFAP in TBI patients with polytrauma when compared to TBI patients alone, which suggests GFAP is a relatively specific marker for astrocytic injury, astrogliosis, or blood-brain barrier damage after TBI.[8]

CASE PRESENTATION

One subject, who received the highest OP exposure during training, is the focus of this Information Review Board (IRB) approved work. The volunteer was a 20-year-old male proficient in English and reported no prior history of TBI or blast exposure. The subject reported approximately (6–7 hours) of sleep daily.

TIMELINE

The subject was part of a breacher training team that completed a 2-week explosive entry course, identical to the pilot study.[2] The course included 3 classroom days and 9 days of practical training, held in the morning, afternoon, and evening sessions. Blast exposure occurred on five of the nine practical training days, with multiple (4–36) exposures over the course of each day. Individual exposure varied on any given blast day because of the characteristics of the training. Variability included changes in the surrounding structural environment, type/strength/number of explosive charges, and subject position relative to the initiation of the blast. The protocol presented here included structural and functional magnetic resonance imaging (MRI), blood sampling, and neurocognitive performance assessment. Testing was conducted before, during, and following course days. Neurocognitive data were incomplete and not assessed for this subject.

DIAGNOSTIC ASSESSMENTS

Serum Sample Collection Procedure

Blood samples were drawn at multiple time points during the study and serum was processed in line with previous works.[3,4] Serum samples were analyzed for concentration levels (ng/mL) of GFAP, UCH-L1, and SBDP150. Seven pre-exposure control blood samples were obtained from the volunteer in the 3 days before the first blast exposure to determine baseline biomarker concentration. Fourteen twice-daily (morning and afternoon) blood samples were obtained from the individual during the breacher course days. The first exposure sample was collected in the afternoon following completion of the first day of blast exposure. The final samples, collected once per day, were collected 1 day and 7 days following completion of the course, respectively.

Brain Biomarkers GFAP, UCH-L1, and SBDP150 Sandwich ELISAs

The GFAP enzyme-linked immunosorbent assay (ELISA) and calpain-generated SBDP150 sandwich ELISA (version-2) were performed in a manner described in the pilot study.[2] UCH-L1 levels in serum were measured using a UCH-L1 sandwich ELISA version-1b modified from a protocol previously reported.[9,10]

Self-reported Symptoms

A symptoms inventory was completed daily to evaluate the presence of 32 symptoms consistent with mTBI (similar to the NSI and Rivermead scales) as well as the degree to which subjects considered these symptoms problematic. This inventory was administered a total of 14 times; three sessions before blast exposure to obtain a baseline, one on each of the 9 practical days, and at 1 and 7 days following completion of the course, respectively. Symptoms were reported on a scale of 0–4 (0, not experienced at all; 4, a severe problem affecting performance).

MRI

MRI was conducted using a Philips 3T scanner equipped with multidirectional diffusion-weighted imaging, in-line blood-oxygen-level-dependent (BOLD) imaging capabilities, and an Invivo ESys fMRI unit. Specific acquired MRI sequences include 3D T1-weighted, axial and coronal T2-weighted, axial fluid attenuation inversion recovery, axial susceptibility weighted imaging, diffusion-weighted imaging in 32 directions for calculation of diffusion tensor imaging maps, and BOLD imaging during administration of the N-back working memory paradigm (fMRI). A version of the N-back task working memory paradigm identical to that used in the study on repeated exposure to LLB in U.S. Marine Corps Breachers was used. The volunteer underwent an initial MRI scan 1 week before the first blast exposure and follow-up scans 1 day and 7 days following completion of the course, respectively.

Blast OP

The subject was instrumented with personal protective equipment (helmet, etc.) identical to that worn in the field. On the sides of the helmet were two interconnected MicroDAS/piezo-resistive pressure transducer modules. Recorded measures on the breacher for each explosive breaching training event were peak incident pressure and incident impulse (pressure

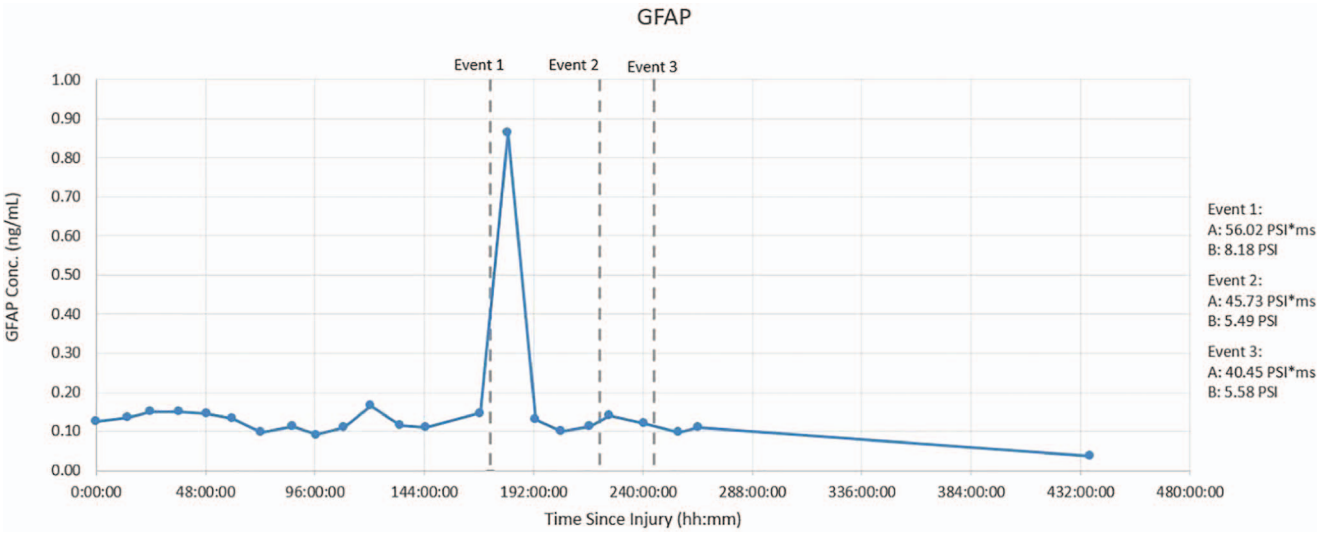

FIGURE 1. GFAP profile and blast exposure for the volunteer. Biomarker profile over time before, during, and after breacher explosive entry training. Serum biomarker concentrations (in ng/mL) were plotted against pretraining (time points 1–7), explosive exposure (time points 8–21), and recovery (22–23, 30). GFAP (glial fibrillary acidic protein) levels are shown; peak pressure events are annotated when >4 psi on the exposure days along with DCI. Note—*vertical dashed lines* indicate blast days with peak pressures >4 psi (peak and DCI reported). The gap for the last time point indicates a 7-day recovery period. The *shaded horizontal box* (95% CI of baseline) with a solid line through the middle (mean) illustrates when GFAP deviated substantially from expected values.

as a function of time). Daily cumulative impulse and total cumulative impulse were calculated.

FINDINGS

Brain Protein Biomarker Levels

Baseline biomarker levels are established for the subject by averaging the seven first time points (before blast exposure) and calculating the mean, standard deviation, and 95% confidence interval (CI) for each participant. Figure 1 illustrates the GFAP biomarker profile plotted across time with a 95% CI of baseline levels. Exposure includes all data points after the first blast exposure and before the recovery period. The vertical dotted lines in the figure represent the three blast exposure days when daily peak OP exceeded 4 psi. The peak pressures are identified next to the line. A stable baseline GFAP concentration (0.13 ng/mL) is seen with a single time point peak of 0.87 ng/mL during the exposure period for the subject. The spike in subject's GFAP occurs right after a maximum daily peak OP of 8.2 psi; the highest peak OP observed during the training.

Blast Exposure and Biomarker Trends

The volunteer had exposure to multiple pressure events greater than 4 psi; GFAP was associated with an acute surge for the subject (from 0.13 to 0.87 ng/mL). Analysis of daily cumulative impulse (DCI) and maximum daily peak pressure (peak pressure) are plotted against GFAP biomarker serum levels. The high daily level of GFAP for subject appeared to be associated with the highest same day peak pressure. Of critical importance is that the GFAP spike for subject occurred on the training day with the highest observed peak OP of 8.2 psi, greater than twice the typical standard used for safety.

In Figure 2, elevated daily UCH-L1 levels for the subject are observed, it appears UCH-L1 spikes follow blast exposure. SBDP150 analysis was also performed but an unstable baseline was identified and was unreliable for evaluation in this work.

The observation of an increase in GFAP in conjunction with the highest peak OP exposure suggests the existence of a relationship between this neurotrauma biomarker and blast OP exposure for the subject.

Self-reported Symptoms

The subject did not report any symptoms during the baseline period. During the training period, an increase in sleep disturbance was reported throughout the study. This symptom appeared after each of the blast days, including the three OP exposures that were greater than 4 psi. After the highest blast was received (8.2 psi), the subject also reported dizziness, fatigue, irritability/anger, and frustration/impatience. All ratings were low (1 on 0–4 scale) but had not existed in baseline reporting. No symptoms were reported during the recovery period.

Neuroimaging Results

All structural MRI images were evaluated by a clinical neuroradiologist and were found to be within normal range while using established methodologies.[11,12]

Follow-up/Outcomes

N/A.

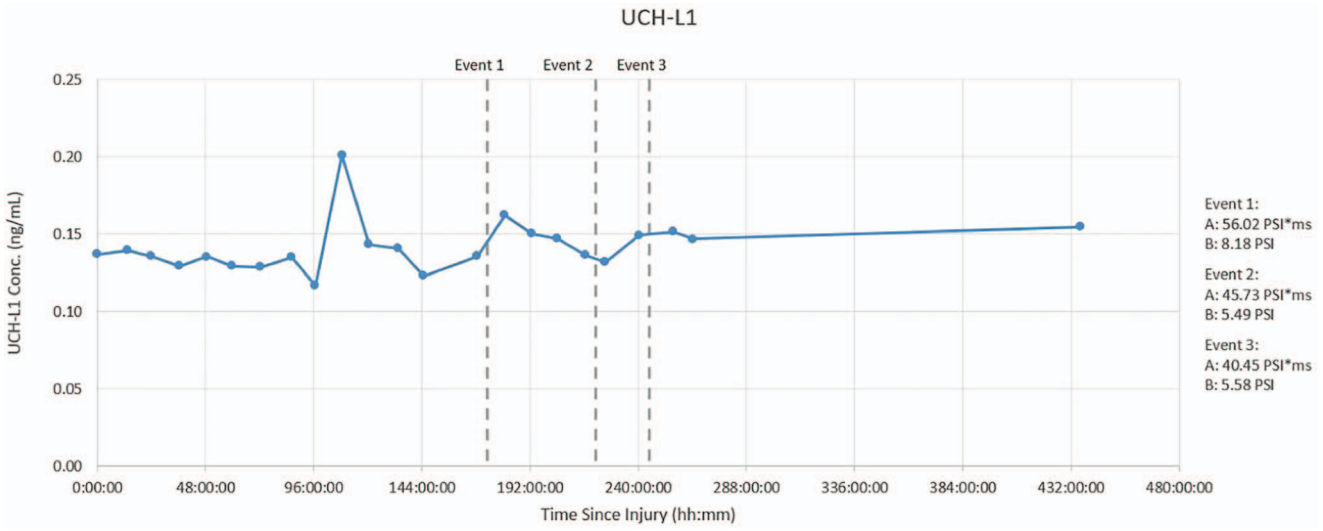

FIGURE 2. UCH-L1 profile and blast exposure for the volunteer. Biomarker profile over time before, during, and after breacher explosive entry training. Serum biomarker concentrations (in ng/mL) were plotted against pretraining (time points 1–7), explosive exposure (time points 8–21), and recovery (22–23). UCH-L1 (ubiquitin C-terminal hydrolase-L1) levels are shown; peak pressure events recorded are annotated when >4 psi on the exposure days along with DCI. Note—*vertical dashed lines* indicate blast days with peak pressures >4 psi (peak and DCI reported). The gap for the last time point indicates a 7-day recovery period. Transparent horizontal boxes (95% CI of baseline) with a solid line through the middle (mean) make it clear to see when biomarkers deviated substantially from expected values.

DISCUSSION

This case study demonstrated an association between high OP exposure and high level of neurotrauma biomarker in an individual. These biomarker measures do not have a threshold for a diagnosable concussion/mTBI injury and elevations resolve after exposures cease. Given that this supports a neurological insult as a result of blast OP, the question of concern is the deleterious effect of repetitive subconcussive events, similar to that seen in direct head impact. Future research should focus on the longitudinal collection of similar, controlled data streams from an adequate sample of personnel involved in regular breacher training. A related concern regarding this occupational group is that observed peak pressures exceeded the 4 psi threshold determined by SSD calculations. These high peak pressures represent an even higher impulse load, which is the energy insult that the human body will absorb as a result of blast exposure. It is recommended that the method of determining SSD for a given explosive weight is reviewed to account for the multiple reflective surfaces in a complex urban environment.

Sensitive and specific objective markers of mTBI remain elusive. Blood-brain protein markers, such as GFAP and UCH-L1, have been promising in this regard. The pilot study showed an individual with biomarker "spikes" after exposure to blast.[2] In this iteration, the individual characterized showed a single time point peak in GFAP (0.87 ng/mL) after exposure to a blast OP twice the typical level used for safety, but not across the other two exposure levels that exceed 4 psi. This maximal GFAP concentration was in the range of levels observed for diagnosed mTBI (0.5–1.1 ng/mL).[3] The occurrence in time of this GFAP peak after the highest peak OP exposure suggests a "blast dose" association, especially given the co-occurrence of symptom reporting. A quantitative dose-response or preceding cumulative dose-response relation requires further investigation. It may be that the association is categorical rather than linear, with GFAP elevations observed after a threshold of exposure is crossed rather than in direct relation to OP magnitude. The presence of an accepted mechanism of insult to the brain—blast OP—and any one objective measure of neurological disruption, such as a blood-brain protein biomarker elevation, is a concern that warrants serious evaluation of the cumulative effects of repetitive insult. Furthermore, this work adds to the growing support that a subset of the population is, for reasons not yet understood, more susceptible to blast effects than others.

Preliminary studies have observed alterations in white matter and cortical structural MRI measure in a cohort of breachers with 7 to 21 years of prior blast exposure that were not seen in a group of less experienced students attending a 2-week breacher course.[13] Although the present study yielded a null neuroimaging result, these data will be available for future comparative analyses.

Previous evidence of the deleterious effect of blast OP on the brain has been demonstrated in a pilot study with a small sample size and with heterogeneous distribution across a proportion of exposed individuals.[2] The case study here manifested symptomologies and blood markers consistent with prior work.[2]

Importantly, this work contributed to a growing body of literature that seems to indicate OP blasts (<4 psi) are unlikely to have deleterious effects on performance and health in young

individuals,[12] and that when exposures exceed 4 psi and become moderate, blood-based biomarkers become detectable in a proportion of the sample population.[2] Though understanding blast effects is far from complete, it seems that moderate blasts can impact some individuals measurably and that LLB effects, to whatever extent they exist, are more subtle and difficult to identify with current methodologies.

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AUTHOR DISCLOSURE STATEMENT

KKWW owns stock of Banyan Biomarkers Inc.

DISCLOSURE

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