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Hippocampal vulnerability and subacute response following varied blast magnitudes

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HIGHLIGHTS

- Low, moderate and high blast pressures (69, 97 and 165 kPa respectively) were used to establish molecular injury thresholds.
- Gene expression using Real Time PCR and protein expression using immunohistochemistry were performed at 7 days post blast.
- GLAST gene expression was up regulated in the low pressure group but down regulated in the high pressure group.
- Map2k1 and mechanosensitive protein Piezo 2 expression was increased at moderate blast level.
- Neuroprotection (BDNF and GDNF) was observed with low and moderate blast levels.

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ABSTRACT

Clinical outcomes from blast neurotrauma are associated with higher order cognitive functions such as memory, problem solving skills and attention. Current literature is limited to a single overpressure exposure or repeated exposures at the same level of overpressure and is focused on the acute response (<3 days). In an attempt to expand the understanding of neuropathological and molecular changes of the subacute response (7 days post injury), we used an established rodent model of blast neurotrauma. Three pressure magnitudes (low, moderate and high) were used to evaluate molecular injury thresholds. Immunohistochemical analysis demonstrated increased cleaved caspase-3 levels and loss of neuronal population (NeuN+) within the hippocampus of all pressure groups. On the contrary, selective activation of microglia was observed in the low blast group. In addition, increased astrocytes (GFAP), membrane signal transduction protein (Map2k1) and calcium regulator mechanosensitive protein (Piezo 2) were observed in the moderate blast group. Results from gene expression analysis suggested ongoing neuroprotection, as brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF) and Mn and CuZn superoxide dismutases (SOD) all increased in the low and moderate blast groups. Ongoing neuroprotection was further supported by increased SOD levels observed in the moderate group using immunohistochemistry. The gene expression level of glutamate aspartate transporter (GLAST) was upregulated in the low, but downregulated in the high blast group, while no changes were found in the moderate group. Overall, the data shown here provides evidence of a diverse neuroprotective and glial response to various levels of blast exposure. This mechanistic role of neuroprotection is vital in understanding ongoing cellular stress, both at the gene and protein levels, in order to develop interventional studies for the prognosis of injury.

1. Introduction

Over 25% of the Veterans returning from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) are suffering from closed head injuries due to blast overpressure (BOP) exposure [1–4]. Moreover, the incidence of civilians exposed to an explosion is becoming more prevalent [1]. Blast induced neurotrauma (BINT) is the second most frequent injury that results from blast exposure, with amputations being the first [1]. Clinical reports have indicated the development of cognitive associated disorders following BOP exposure. The majority of these disorders are associated with anxiety, attention deficits, memory issues and impaired/altered problem solving skills [2,3]. Overlapping symptoms with other forms of trauma, such as non-blast related traumatic brain injury

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(TBI) and post-traumatic stress disorder (PTSD) have confounding effects on differential diagnosis. Animal models of blast injuries have been established to investigate injury mechanisms from BOP exposure and its subsequent neurological impairments. Most published studies are limited to a single BOP exposure or repeated exposures at the same magnitude of overpressure. Information on the molecular changes within the hippocampus in response to varied levels of overpressure at a subacute stage (3–7 days post injury) is still unknown.

Preclinical studies have shown preliminary assessments of injury mechanisms associated with morbidity related to BINT [5,6]. However, a detailed mechanism and prognosis of injury is poorly understood. Animal studies have shown acute (<3 days) astrocyte activation, neurodegeneration and neurochemical changes following BOP exposure in various regions of the brain, such as the hippocampus, cortical regions, amygdala and cerebellum [5–7]. However, there is a gap in reported subacute and chronic outcomes and neuropathology following blast. Deciphering the relationship between molecular mediators of neuropathology (oxidative stress and excitotoxicity) and cognitive impairment in relation to blast magnitude will provide the necessary data for the development of new remedies for BINT. A recent study examining animals exposed to single and repeated blasts indicated a role of tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin-6 (IL-6) in the ventral hippocampus and amygdala [8]. However, details of affected gene regulation and protein expression within the hippocampus are yet to be understood at a subacute stage. In addition, discovery of mechanosensitive proteins that regulate cation channels, Piezo 1 and Piezo 2, are of potential interest for understanding cellular homoeostasis with respect to pathological cascades triggered by BINT.

In this study, a comprehensive evaluation of hippocampal gene expression for oxidative stress (Mn and CuZn SODs), glutamate associated receptor regulation for excitotoxicity (GLAST), signal transduction (MAP2k1, Piezo 2) and anti-inflammation (IL-3, BDNF) was performed utilizing real-time polymerase chain reaction (RT-PCR) for pathways that are associated with blast exposure. Immunohistochemistry was employed to validate protein changes within resultant cascades of the genes evaluated. Collectively, our goal is to identify the pressure thresholds which elicit protein expression or inhibition, which could reveal targets for drug development.

2. Methods

2.1. Experimental procedures

2.1.1. Animals and testing parameters

The Virginia Tech Institutional Animal Care and Use Committee approved the experimental protocols described herein. Prior to all experiments, male Sprague Dawley rats (~250 g, Harlan Labs, San Diego) were acclimated to a 12 h light/dark cycle with food and water provided ad lib. The shock front and dynamic overpressure was generated using a custom-built advanced blast simulator (ABS) (200 cm × 30.48 cm × 30.48 cm) consists of a driving compression chamber attached to a rectangular transition and testing chamber with an end wave eliminator (ORA Inc. Fredericksburg, VA) located at the Center for Injury Biomechanics of Virginia Tech University. A passive end-wave eliminator (EWE) was installed at the venting end of the ABS, which minimizes the shock-wave outflow by means of a grill or perforated plate system. Patterns in the EWE were created to mirror reflected shocks and rarefactions, which tend to 'cancel' each other and diminish unwanted effects within the test section. A peak static overpressure was produced with compressed helium and calibrated acetate sheets (Grafix Plastics, Cleveland, OH) [9].

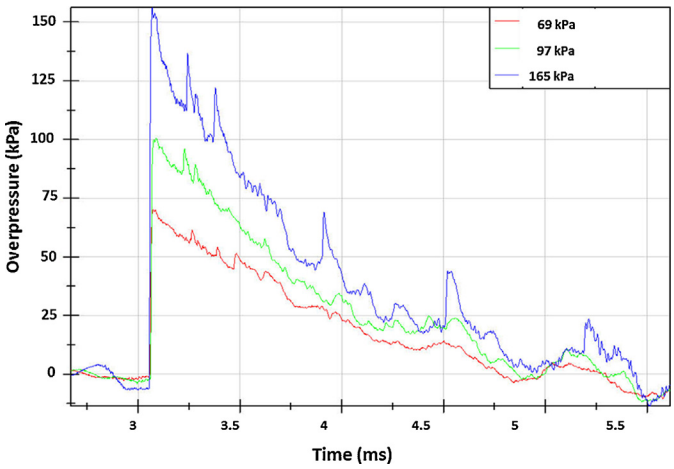

Fig. 1. Representative pressure profiles of low (69), moderate (97) and high (165) blast.

Pressure measurements were collected at 250 kHz using a Dash 8HF data acquisition system (Astro-Med, Inc., West Warwick, RI). The animals were briefly anesthetized with 3% isoflurane before being placed in a rostral cephalic orientation toward the shock front. Animals were randomly placed within a pressure group (n = 5/group) and were exposed to a single incident pressure profile resembling a 'free-field' blast exposure with a positive duration of 2.5 ms [9]. Peak pressures of 69 (low), 97 (moderate), 165 (high) kPa or the sham (anesthetized with 3% isoflurane but did not experience the overpressure) were used to gain insight into the molecular changes occurring at various blast levels and to identify distinct responses in the injury pathways (Fig. 1).

In order to obtain subacute outcome measurements, animals were anesthetized with isoflurane (3%) and euthanized by transcardial perfusion using ice-cold phosphate buffered saline (PBS) containing 6 unit/ml heparin seven days following blast. After extraction, the brain was separated into two hemispheres. The hippocampus of the left hemisphere was dissected, immediately frozen on dry ice and stored at −80 °C until analysis. The right hemisphere was cryopreserved in 30% sucrose solution for 24 h and processed in Tissue-Tek® optimal cutting temperature (OCT) embedding medium (Sakura Finetek USA, Inc., Torrance, CA). Serial 20-μm-thick sagittal sections were prepared for immunohistochemistry [9].

2.1.2. Real time polymerase chain reaction (RT-PCR)

RT-PCR was used to measure gene expression from tissue samples relative to the internal housekeeping gene control. The total RNA was extracted from the tissues with Trizol (Invitrogen, Carlsbad, CA) following the manufacturer's protocol and homogenized using a Branson Ultrasonicator (Fisher Scientific, Hampton, NH). The RNA was then purified using RNeasy MinElute Cleanup kit (Qiagen, Valencia, CA). The RNA concentration and purity was determined using a spectrophotometer through UV absorbance at 260 nm and 280 nm. Using random primers, reverse transcriptase was used to convert the mRNA to a cDNA template using a thermal cycler (Mastercycler Gradient, Eppendorf, Hauppauge, NY). For PCR analysis, cDNA equivalent to 40 ng of total RNA was used. Specific primer pairs were used, including brain-derived neurotrophic factor (BDNF), glial-cell derived neurotrophic factor (GDNF), glial fibrillary acidic protein (GFAP), glutamate aspartate transporter (GLAST), interleukin-3 (IL-3), mitogen activated protein kinase kinase 1 (MAP2k1), Piezo 2 and superoxide dismutases (CuZnSOD