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BLAST INDUCES OXIDATIVE STRESS, INFLAMMATION, NEURONAL LOSS AND SUBSEQUENT SHORT-TERM MEMORY IMPAIRMENT IN RATS

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Abstract—Molecular and cellular mechanisms of brain injury after exposure to blast overpressure (BOP) are not clearly known. The present study hypothesizes that pro-oxidative and pro-inflammatory pathways in the brain may be responsible for neuronal loss and behavioral deficits following BOP exposure. Male Sprague–Dawley rats were anesthetized and exposed to calibrated BOP of 129.23 ± 3.01 kPa while controls received only anesthesia. *In situ* dihydroethidium fluorescence staining revealed that BOP significantly increased the production of reactive oxygen species in the brain. In addition, real-time reverse transcriptase-polymerase chain reaction, immunofluorescence staining and enzyme-linked immunosorbent assay demonstrated a significant up-regulation of mRNA and protein expressions of pro-inflammatory mediators, such as interferon-γ and monocyte chemoattractant protein-1, in brains collected from BOP-exposed animals compared with the controls. Furthermore, immunoreactivity of neuronal nuclei in brains indicated that fewer neurons were present following BOP exposure. Moreover, novel object recognition paradigm showed a significant impairment in the short-term memory at 2 weeks following BOP exposure. These results suggest that pro-oxidative and pro-inflammatory environments in the brain could play a potential role in BOP-induced neuronal loss and behavioral deficits. It may provide a foundation for defining a molecular and cellular basis of the pathophysiology of blast-induced neurotrauma (BINT). It will also contribute to the development of new therapeutic approaches selectively targeting these pathways, which have great potential in the diagnosis and therapy of BINT.

Key words: blast-induced neurotrauma, oxidative stress, inflammation, neuronal loss, novel object recognition, short-term memory impairment.

INTRODUCTION

According to the Armed Forces Health Surveillance (2013), over 80,000 cases of US Armed Forces service members diagnosed as mild or severe traumatic brain injury (TBI) during the surveillance period between 2000 and 2012 were Veterans returning from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). These individuals have suffered traumatic injuries due to blast overpressure (BOP) exposure (Warden, 2006; Hoge et al., 2008; Nampiaparampil, 2008; Bazarian et al., 2009; Cernak and Noble-Haeusslein, 2010; Elder et al., 2010). In addition, BOP exposure has been reported to cause polytrauma in the military population (e.g. OIF/OEF Veteran), indicating multiple traumatic injuries in other organs including the lungs, intestines, eyes, and ears (Cave et al., 2007; Wani et al., 2009; Morley et al., 2010; Smith, 2011). Although blast-induced TBI is a notable military health issue, it is also a menace to civilians who experienced BOP by explosion without protective equipment, which possesses a high risk of blast-induced TBI (Nampiaparampil, 2008; Cernak and Noble-Haeusslein, 2010; Elder et al., 2010; Hicks et al., 2010).

Blast-induced TBI is the second most common cause of injuries from BOP, next to amputations (Rauh et al., 2013). Pre-clinical and clinical reports have indicated the development of cognitive-associated disorders following BOP exposure. Majority of these disorders are associated with anxiety, attention deficits, memory issues and impairments in problem-solving skills (Elder and Cristian, 2009; Ruff et al., 2009; Cernak and Noble-Haeusslein, 2010; Hicks et al., 2010; Rosenfeld and Ford, 2010). Overlapping symptoms with other forms of trauma such as impact-related TBI and post-traumatic stress disorder has confounding effects on diagnosis (Warden, 2006; Hoge et al., 2008; Nampiaparampil, 2008; Elder et al., 2010).

Animal models of blast injury are currently under investigation in order to understand the molecular and

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Abbreviations: BINT, blast-induced neurotrauma; BOP, blast overpressure; BSA, bovine serum albumin; C_T, threshold cycle; DHE, dihydroethidium; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN-γ, interferon-γ; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; NADPH, nicotinamide adenine dinucleotide phosphate; NeuN, neuronal nuclei; NOR, novel object recognition; OIF, Operation Iraqi Freedom; OEF, Operation Enduring Freedom; PBS, phosphate-buffered saline; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymer chain reaction; TBI, traumatic brain injury; TNF-α, tumor necrosis factor-α; T1, trial 1; T2, trial 2.

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cellular mechanisms of blast-induced neurotrauma (BINT) leading to cognitive impairment. Many pre-clinical studies have proposed different mechanisms of injury associated with morbidity related to BINT (Bhattacharjee, 2008; Belanger et al., 2009; Säljö et al., 2010; Bolander et al., 2011; Cernak et al., 2011; Leonardi et al., 2011). Various approaches have been applied to elucidate astrocyte activation, neurodegeneration and neurochemical changes following BOP exposure in various regions of the brain such as hippocampus, cortical regions, amygdala and cerebellum (Cernak et al., 2001, 2011; Hoge et al., 2008; Aarabi and Simard, 2009; Elder and Cristian, 2009; Kocsis and Tessler, 2009; Kamnaksh et al., 2011; Luethcke et al., 2011; Risling et al., 2011; Sajja et al., 2012, 2013; VandeVord et al., 2012). However, a detailed mechanism and prognosis of injury is poorly understood yet.

Pro-oxidative and pro-inflammatory environments in the brain have been implicated in the onset and progression of neurological and psychiatric disorders (Martino et al., 2002; Allan and Rothwell, 2003; Lucas et al., 2006; Uttara et al., 2009; Amor et al., 2010; Glass et al., 2010; Tansey and Goldberg, 2010; Najjar et al., 2013). Reactive oxygen species (ROS) is a significant contributor to neurotoxic consequences mediated by oxidative stress. ROS, such as hydrogen peroxide and superoxide anion, are small and reactive oxygen-derived molecules regarded as essential participants in neurotoxicity and neurodegeneration (Block et al., 2007; Lull and Block, 2010). Thus, excessive ROS production interferes with cell signaling and the regulation of important physiological conditions. In addition, pathophysiologcal conditions involving ROS generation causes cell death and degeneration by up-regulation of pro-inflammatory mediators leading to severe or prolonged inflammation (Block and Hong, 2007; Yang et al., 2011; Salim et al., 2012). Few studies have shown the role of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interferon-γ (IFN-γ) in various regions of the brain including the ventral hippocampus and amygdala following BOP exposure (Kamnaksh et al., 2011; Kovesdi et al., 2011; Kwon et al., 2011; Dalle Lucca et al., 2012). ROS generation following BOP exposure was also observed in previous studies (Cernak et al., 2000; Abdul-Muneer et al., 2013). However, the role of pro-oxidative and pro-inflammatory pathways in BINT remains largely unknown.

In the present study, a time-course evaluation of hippocampal pro-oxidative/inflammatory environments with subsequent neuronal changes was examined at an acute stage following BOP exposure. In addition, behavioral assessment for short-term memory impairment was performed using novel object recognition (NOR) paradigm.

EXPERIMENTAL PROCEDURES

Animals and BOP exposure

The Virginia Polytechnic Institute and State University (Virginia Tech) Institutional Animal Care and Use Committee approved experimental protocols described herein. Prior to all experiments, animals were acclimated for at least three days (12 h light/dark) and food and water provided *ad libitum*. Male Sprague–Dawley rats weighing approximately 250 g (n = 5 per group) were briefly anesthetized with isoflurane (3%) and positioned inside the shock tube with a rostral cephalic orientation toward the shock wave. The blast shock front and dynamic overpressure were generated by a custom-built blast simulator (ORA Inc., Fredericksburg, VA; Fig. 1A) consisting of a driving compression chamber paired with a rectangular test section and end wave eliminator located at the Center for Injury Biomechanics at Virginia Tech. A peak static overpressure was produced with compressed helium and calibrated acetate sheets (Grafix Plastics, Cleveland, OH). The advanced blast simulator generated a single (free field) pressure insult. Animals were exposed to a peak overpressure of 129.23 ± 3.01 kPa (18.74 ± 0.44 psi) for 2.5 milliseconds duration; sham animals did not experience the overpressure. The applied intensity was based on previous studies due to the visible molecular alterations of brain injury (Long et al., 2009; VandeVord et al., 2012; Abdul-Muneer et al., 2013). Fig. 1B provides a representative pressure profile of the shock wave generated from the blast simulator. Time profile is determined with a piezoelectric sensor axial to the blast pressure source and recorded at 250 kHz (per channel) as described previously (Sajja et al., 2012, 2013; VandeVord et al., 2012).

Tissue collection and preparation

Separate animals at 4, 24, 48 h and 2 weeks following BOP exposure (n = 5 per group) were briefly

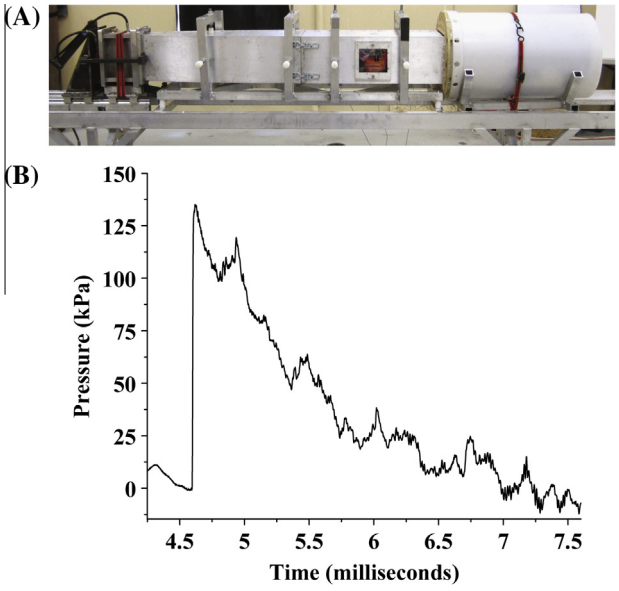

Fig. 1. Blast simulator and pressure profile of the shock wave. Pressure profile of a single (free field) overpressure was generated from the advanced blast simulator. (A) The advanced blast simulator located at Virginia Tech. (B) Representative pressure profile of the shock wave.