Free Radical Biology and Medicine 60 (2013) 282–291

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contrbution

Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast

P.M. Abdul-Muneer [a], Heather Schuetz [a], Fang Wang [c], Maciej Skotak [c], Joselyn Jones [b], Santhi Gorantla [a], Matthew C. Zimmerman [b], Namas Chandra [c], James Haorah [a,*]

[a] Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198, USA
[b] Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, NE 68198, USA
[c] Department of Mechanical and Materials Engineering, University of Nebraska at Lincoln, Lincoln, NE 68588, USA

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form
10 January 2013
Accepted 23 February 2013
Available online 4 March 2013

Keywords:
Mild traumatic brain injury
Blood–brain barrier
Oxidative stress
Perivascular unit
Neuroinflammation
Primary blast
Free radicals

ABSTRACT

We investigate the hypothesis that oxidative damage of the cerebral vascular barrier interface (the blood–brain barrier, BBB) causes the development of mild traumatic brain injury (TBI) during a primary blast-wave spectrum. The underlying biochemical and cellular mechanisms of this vascular layer-structure injury are examined in a novel animal model of shock tube. We first established that low-frequency (123 kPa) single or repeated shock wave causes BBB/brain injury through biochemical activation by an acute mechanical force that occurs 6–24 h after the exposure. This biochemical damage of the cerebral vasculature is initiated by the induction of the free radical-generating enzymes NADPH oxidase 1 and inducible nitric oxide synthase. Induction of these enzymes by shock-wave exposure paralleled the signatures of oxidative and nitrosative damage (4-HNE/3-NT) and reduction of the BBB tight-junction (TJ) proteins occludin, claudin-5, and zonula occluden 1 in the brain microvessels. In parallel with TJ protein disruption, the perivascular unit was significantly diminished by single or repeated shock-wave exposure coinciding with the kinetic profile. Loosening of the vasculature and perivascular unit was mediated by oxidative stress-induced activation of matrix metalloproteinases and fluid channel aquaporin-4, promoting vascular fluid cavitation/edema, enhanced leakiness of the BBB, and progression of neuroinflammation. The BBB leakiness and neuroinflammation were functionally demonstrated in an in vivo model by enhanced permeativity of Evans blue and sodium fluorescein low-molecular-weight tracers and the infiltration of immune cells across the BBB. The detection of brain cell proteins neuron-specific enolase and S100β in the blood samples validated the neuroastroglial injury in shock-wave TBI. Our hypothesis that cerebral vascular injury occurs before the development of neurological disorders in mild TBI was further confirmed by the activation of caspase-3 and cell apoptosis mostly around the perivascular region. Thus, induction of oxidative stress and activation of matrix metalloproteinases by shock wave underlie the mechanisms of cerebral vascular BBB leakage and neuroinflammation.

Published by Elsevier Inc.

Introduction

Traumatic brain injury (TBI) is characterized by physical brain injury as a result of acceleration–deceleration, resulting frequently from impact with an immobile object, that often leads to cognitive deficits and impairment of behavior. Unlike casualties suffered from moderate to severe TBI, victims diagnosed with mild TBI (mTBI) remain conscious, and typical symptoms include headache, confusion, dizziness, memory impairment, and behavioral changes. U.S. soldiers exposed to blast-wave pressure and combat experiences without any physical brain injury during Middle East wars are commonly diagnosed with mild TBI and posttraumatic stress disorder [1]. Mild TBI is the most frequent form of trauma among deployed military populations [2]. In recent military conflicts, the repeated exposure to low levels of blast overpressure from improvised explosive devices is believed to account for the majority of the mTBI's. Ironically, most of these soldiers exposed to low-intensity blast remain conscious, and many of these soldiers are frequently redeployed in the war zone without proper diagnosis. This in turn puts these military personnel in danger of experiencing consecutive multiple blast exposures aggravating an already existing medical condition [3,4]. These subjects undergo severe mental stress and often become misusers of alcohol and other drugs of abuse [5,6], and thereby the chances of mental health complications such as

* Corresponding author. Fax: +402 559 8922.
E-mail address: jhaorah@unmc.edu (J. Haorah).

0891-5849/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.freeradbiomed.2013.02.029

posttraumatic stress disorder (PTSD) increase in the long term [7]. It seems that there is a strong overlap between symptoms of chronic mTBI and PTSD among many veterans reexposed to multiple blasts of wave pressure, which has become a major challenge for the military healthcare system. Psychological and physiological stress by repeated blast-wave exposure is believed to contribute significantly to the development of PTSD in chronic mTBI, resulting in alterations in cognitive behaviors [4,8]. These findings are further supported by recent demonstration in an animal model that low levels of shock wave can cause cognitive deficits in short-term learning and memory [9].

Intriguingly, epidemiological findings indicate that disruption of the blood–brain barrier (BBB) is involved in shock-wave-induced mTBI and neurological disorders such as PTSD [10]. However, such cohort studies lack the understanding of the underlying mechanisms. Thus, uncovering the molecular, biochemical, and cellular mechanisms of shock-wave–brain interactions leading to mTBI requires a careful investigation. We have shown that cerebral vascular integrity (the BBB) is very sensitive to oxidative stress during substance abuse [11,12]. Ensuing oxidative damage of the BBB leads to neuroinflammation and neuronal degeneration [13–15]. Understanding the underlying molecular and biochemical mechanisms will help us define the characteristic biomarkers of cerebral vascular injury and formulate a preventive strategy to mitigate the adverse acute effects of blast exposure and related chronic neurological complications. This includes the identification of the blast-simulated shock-wave range (shock-wave frequencies) that causes such physiological deficits and the types of mechanical or biochemical injury.

Blast injuries are classified as primary (pure blast), secondary (interaction with shrapnel or fragments), tertiary (impact with environmental structures), and/or quaternary (toxic gases) [16–18]. The 15-point Glasgow Coma Scale [19] defines severity of traumatic brain injury as mild TBI (13–15), moderate TBI (9–12), severe TBI (3–8), and vegetative state TBI (3). Mild TBI considered in this work is defined as loss of consciousness for less than 24 h [20]. At the initial stage of this study we identified the pressure range of 90–150, 150–230, and 230–350 kPa as corresponding to the mild, moderate, and severe TBI in a rodent model. All tests were conducted using a 9-in. square cross-section shock tube at the U.S. Army–University of Nebraska at Lincoln Center for Trauma Mechanics facility (Fig. 1). The detailed description of the shock-wave generator, the blast-wave spectral content, and the numerical models of skull and brain responses to blast loading and corresponding transition of blast energy are provided elsewhere [21–23]. We hypothesized that induction of oxidative stress by single (one time only shock-wave pressure exposure) and repeated (more than one shock-wave pressure exposure on the same animal) exposures to low-intensity blast overpressure initiates cerebral vascular injury (BBB damage) and neuroinflammation, which are accompanied by the release of mTBI-specific biomarkers into the blood circulation. Thus, the disruption of the barrier interface (BBB) is a key event in the acute phase of mTBI development. We demonstrate that the induction of free radical-generating enzymes, oxidative damage markers, BBB leakage, perivascular regulation by matrix metalloproteinases, and fluid channel activator aquaporin-4 ultimately leads to neuroinflammation. These pathological processes can be manifested as long-term neurological disorders.

Materials and methods

Reagents

The primary antibodies rabbit anti-NADPH oxidase 1 (NOX1), anti-inducible nitric oxide synthase (iNOS), anti-4-hydroxynonenal (4-HNE), anti-claudin-5, anti-matrix metalloproteinase 3 (MMP-3), anti-MMP-9, anti-aquaporin-4 (AQP-4), anti-Iba1, anti-caspase-3; mouse anti-GLUT1, anti-3-nitrotyrosine (3-NT), anti-MMP-2; sheep anti-von Willibrand factor (vWF); and goat anti-Iba1 were purchased from Abcam (Cambridge, MA, USA). Mouse anti-occludin antibody was purchased from Invitrogen (Carlsbad, CA, USA); rabbit anti-zonula occluden 1 (ZO-1) was from U.S. Biological (Salem, MA, USA); mouse anti-β-actin was from Millipore (Billerica, MA, USA); rabbit anti-XOX was from Santa Cruz Biotechnology (Santa Cruz, CA, USA); and mouse anti-PDGFR-β was from eBiosciences (San Diego, CA, USA). All secondary Alexa Fluor-conjugated antibodies and Fluo-3 were purchased from Invitrogen. The enzyme-linked immunosorbent assay (ELISA) kits for neuron-specific enolase (NSE) and S100β were from Alpha Diagnostic (San Antonio, TX, USA) and Abnova (Walnut, CA, USA), respectively. Evans blue (EB) and sodium fluorescein (Na-Fl) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

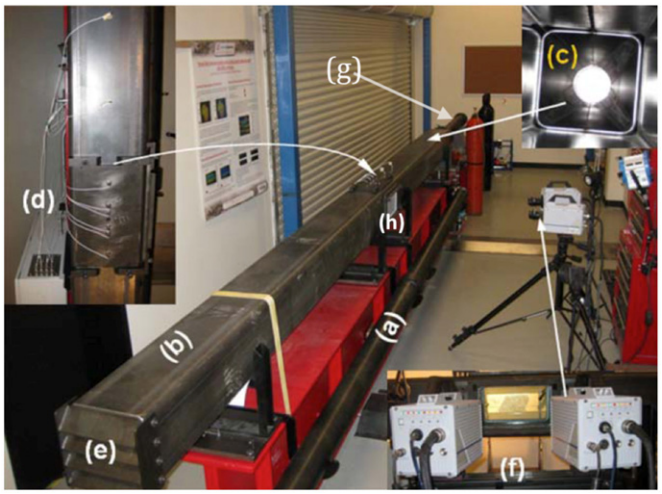

Fig. 1. Blast-wave simulation and testing facility at University of Nebraska at Lincoln. (a) 4-in. cylindrical shock tube; (b) 9-in. square shock tube; (c) transition section; (d) pressure sensor array; (e) adjustable end reflector; (f) Photron SA1.1 high-speed cameras; (g) driver section; (h) test section.

Exposure of animals to primary blast wave

Nine-week-old male Sprague–Dawley rats were purchased and maintained in sterile cages under pathogen-free conditions in accordance with the National Institutes of Health guidelines for the ethical care of laboratory animals and the Institutional Animal Care and Use Committee at the animal facility of the University of Nebraska at Lincoln. First, we determined the effects of various intensities of blasts at 123, 190, 230, and 250 kPa on the severity of brain injury. For this, we exposed 11-week-old rats (6 animals per wave intensity) to this range of blast intensities and we evaluated the blast-induced brain pathology. Second, because 123-kPa (mTBI) intensity did not cause any visible brain injury, we determined the kinetic profile of a one-time 123-kPa intensity blast on the underlying mechanisms of cerebral vascular and brain injuries at 1, 6, 24, and 48 h and 8 days postexposure in the same age group of animals (11-week-old rats). Third, after establishing the time-dependent effect at 6–24 h after 123-kPa exposure, we examined the possible exacerbating effects of repeated exposure to 123-kPa intensity at 24 h. That is, 11-week-old rats (12 rats) were exposed to 123-kPa intensity and then 24 h after the first exposure, 6 animals of the 12 were reexposed to 123-kPa intensity and left for another 24 h, which is termed here as repeated exposure or the 24 hrR group. Animals comprising the control (unexposed), 24-h exposure, and 24 hrR (6 each) groups were euthanized with a ketamine/xylazine mixture. Brain tissues were dissected out, embedded in optimal