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BLAST EXPOSURE CAUSES DYNAMIC MICROGLIAL/MACROPHAGE RESPONSES AND MICRODOMAINS OF BRAIN MICROVESSEL DYSFUNCTION

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Abstract—Exposure to blast overpressure (BOP) is associated with behavioral, cognitive, and neuroimaging abnormalities. We investigated the dynamic responses of cortical vasculature and its relation to microglia/-macrophage activation in mice using intravital two-photon microscopy following mild blast exposure. We found that blast caused vascular dysfunction evidenced by microdomains of aberrant vascular permeability. Microglial/-macrophage activation was specifically associated with these restricted microdomains, as evidenced by rapid microglial process retraction, increased ameboid morphology, and escape of blood-borne Q-dot tracers that were internalized in microglial/macrophage cell bodies and phagosome-like compartments. Microdomains of cortical vascular disruption and microglial/macrophage activation were also associated with aberrant tight junction morphology that was more prominent after repetitive (3X) blast exposure. Repetitive, but not single, BOPs also caused TNFα elevation two weeks post-blast. In addition, following a single BOP we found that aberrantly phosphorylated tau rapidly accumulated in perivascular domains, but cleared within four hours, suggesting it was removed from the perivascular area, degraded, and/or dephosphorylated. Taken together these findings argue that mild blast exposure causes an evolving CNS insult that is initiated by discrete disturbances of vascular function, thereby setting the stage for more protracted and more widespread neuroinflammatory responses. **© 2016 Published by Elsevier Ltd. on behalf of IBRO.**

Key words: blood–brain barrier, two-photon microscopy, neuropathology, microglia, macrophages.

INTRODUCTION

Mild traumatic brain injury (mTBI) from blast exposure is the most common form of neurotrauma experienced by military forces in Iraq and Afghanistan (Owens et al., 2008; Bell et al., 2009). Expanded use of improvised explosive devices and multiple deployments have increased the frequency of exposure, while advances in body armor and battlefield medicine have improved survival. Studies of Veterans with repetitive blast-induced mTBI have demonstrated persistent postconcussive symptoms, as well as extensive structural and functional neuroimaging abnormalities (Mac Donald et al., 2011; Jorge et al., 2012; Petrie et al., 2014). Moreover, the neuropathology of chronic traumatic encephalopathy (CTE) has been reported in Iraq Veterans with a history of blast injury (Omalu et al., 2011; Goldstein et al., 2012; McKee et al., 2013).

Growing evidence indicates that blast exposure is capable of disrupting cortical vessels forming the blood–brain barrier (BBB), provoking microglial/macrophage activation. An initial mechanism of blast-induced vascular disruption is thought to involve physical damage to vessels from mechanical forces leading to oxidative damage and reduced expression of tight junction proteins (Abdul-Muneer et al., 2013). However, the real-time dynamic responses of CNS microvasculature and microglia/macrophages to blast-induced mTBI have not been reported. Non-blast CNS injury

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Abbreviations: BBB, blood–brain barrier; BOP, blast overpressure; CTE, chronic traumatic encephalopathy; DAB, diaminobenzidine; GFP, green fluorescent protein; mTBI, Mild traumatic brain injury; PBS, phosphate-buffered saline; SPF, specific antigen-free.

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approaches have been shown to activate microglia/-macrophages, which mobilize to the site of injury (Nimmerjahn et al., 2004; Roth et al., 2014) recruiting peripheral monocytes and neutrophils to assist in the elimination of damaged tissue (Shechter et al., 2009; London et al., 2011). Depending on the method of injury, BBB disruption can result in either rapid or delayed glial responses (Seiffert et al., 2004; Nimmerjahn et al., 2005). *In vivo* studies of skull depression-induced TBI demonstrate microglia with extended, enlarged, and flattened processes that form confluent sheets over the area of injury (Roth et al., 2014). Focal disruption of vessels with targeted laser pulses also causes microglial migration to the site of injury, where microglia shield injured vessels from the surrounding parenchyma (Nimmerjahn et al., 2005). These *in vivo* models suggest that microglia form barriers at sites of injury, particularly in regions adjacent to the glial limitans.

Accumulating evidence suggests that blast exposure provokes pathophysiological responses that can be distinct from non-blast TBI (Elder et al., 2014). Thus, to better understand the dynamic pathophysiological underpinnings of blast-related mTBI we have used our established murine model of blast-induced mTBI (Huber et al., 2013) to examine the early-occurring dynamic relationships between vascular disruption and microglia/-macrophage responses to mild BOP using real-time *in vivo* two-photon microscopy via a thinned-skull imaging methodology. Thinned skull preparations result in little or no inflammation of the underlying cortex, thereby allowing study of the native response of microglia to blast-induced vascular permeability (Davalos et al., 2005; Xu et al., 2007; Yang et al., 2010). Using this approach, we have found that mild blast exposures that are comparable to those commonly experienced by military service members provoke discrete microdomains of vascular dysfunction and correspondingly discrete microglial/macrophage responses that are associated with aberrant tight junction morphology.

EXPERIMENTAL PROCEDURES

Shock tube

The shock tube was designed to generate shock waves that replicate combat-relevant forces produced by open-field high-explosive detonations (Baker Engineering and Risk Consultants, San Antonio, TX, USA). The characteristics and operational properties of the shock tube are described in detail elsewhere (Huber et al., 2013). Briefly, the shock tube consists of a variable volume driver that controls primary positive peak duration. A dual diaphragm spool distributes the pressure difference between the driver and driven section of the tube across two membranes. The shock tube is activated by rapidly releasing the pressure between the two diaphragms causing rupture of both diaphragms via a remotely controlled high-speed electronic valve. Static pressure measurements were recorded via three side-mounted pressure sensors (PCB Piezoelectronics, Depew, NY, USA) positioned 89 cm upstream, 89 cm downstream, and adjacent to the animal harness. Pressure sensor data were collected at 20 kHz with a National Instruments analog-to-digital data acquisition unit (Austin, TX, USA) and processed using a custom LabVIEW interface (National Instruments, Austin, TX, USA). The end of the shock tube is fitted with an attenuator that reduces ambient transient blast noise to less than 100 decibels and suppresses reflected shock waves.

Animals and blast parameters

As indicated in the Results Section, 3–4-month-old male CX3CR1-GFP$^{+/-}$ ($n = 14$) or wild type C57BL/6 ($n = 80$) mice (Jackson Laboratories) were used for this study. All mice had *ad libitum* access to food and water, were maintained under specific antigen-free (SPF) conditions with a 12/12-h day/night cycle, and housed two– four per cage. All animals were housed and handled in accordance with protocols approved by the Veterans Affairs Puget Sound Health Care System's Institutional Animal Care and Use Committee (IACUC) and all experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiment group sample sizes were based on prior pathologic findings (Huber et al., 2013). All mice were allowed to acclimatize to the animal facility for at least one week prior to blast exposure. In preparation for blast exposure, animals were anesthetized with 2% isoflurane delivered with a non-rebreathing anesthesia machine at a flow rate of 1 L/min oxygen after initial induction with 5% isoflurane. A flexible custom facemask designed to fit over the nose and mouth was attached to the mouse harness and provided anesthesia during blast exposure or sham treatment. Mice were warmed on a heating pad (Gaymar, Orchard Park, NY, USA) while anesthetized, except for the 2–5 min spent in the shock tube (the time required to position the animal, pressurize the driver, deliver blast overpressure [BOP], and remove the animal). To minimize blast-induced head and body motion, the mice were securely mounted using plastic cable ties that attached each limb to a steel frame restraint harness supporting an open ¼-inch rigid mesh. Aiming to reproduce a common occurring in-theater scenario where the military service member is facing the incoming shock wave with the torso turned toward the shock wave front, the animals' ventral body surface was oriented perpendicular with respect to the oncoming blast wave in accordance with well-established methods (Koliatsos et al., 2011). Each BOP-exposed animal was followed by a yoked non-blasted sham control animal that was mounted in the restraint harness and held under anesthesia for the same amount of time as its paired BOP-exposed mouse, hence treatment selection was not randomized. The BOP used in these experiments had a peak static pressure of 15.3 psi (s.e.m. ± 0.25), a positive phase duration of 5.78×10^{-3} s (s.e.m. $\pm 1.31 \times 10^{-4}$), with a resulting impulse of 2.55×10^{-2} psi*s (s.e.m. $\pm 5.34 \times 10^{-4}$), and a shock wave velocity of 1.4 Mach. Mice that were used for Western blotting or immunohistochemistry were immediately removed from the shock tube following blast exposure or sham treatment and placed in a partially heated observation enclosure (37 °C). Animals were observed

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