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Neuroinflammation in primary blast neurotrauma: Time course and prevention by torso shielding

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ABSTRACT

Mechanisms of primary blast injury caused by overpressure are not fully understood. In particular, the presence and time course of neuroinflammation are unknown and so are the signatures of reactive inflammatory cells, especially the neuroprotective versus injurious roles of microglia. In general, chronic microglial activation in the injured brain suggests a pro-degenerative role for these reactive cells. In this study, we investigated the temporal dynamics of microglial activation in the brain of mice exposed to mild–moderate blast in a shock tube. Because, in our previous work, we had found that torso shielding with rigid Plexiglas attenuates traumatic axonal injury in the brain, we also evaluated neuroinflammatory microglial responses in animals with torso protection at 7 days post blast injury. Because of the prominent involvement of the visual system in blast TBI in rodents, activated microglial cells were counted in the optic tract at various time points post-injury with stereological methods. Cell counts (activated microglial cell densities) from subjects exposed to blast TBI were compared with counts from corresponding sham animals. We found that mild–moderate blast injury causes focal activation of microglia in certain white matter tracts, including the visual pathway. In the optic tract, the density of activated microglial profiles gradually intensified from 3 to 15 days post-injury and then became attenuated at 30 days. Torso protection significantly reduced microglial activation at 7 days. These findings shed light into mechanisms of primary blast neurotrauma and may suggest novel diagnostic and monitoring methods for patients. They leave open the question of whether microglial activation post blast is protective or detrimental, although response is time limited. Finally, our findings confirm the protective role of torso shielding and stress the importance of improved or optimized body gear for warfighters or other individuals at risk for blast exposure.

1. Introduction

Blast-induced neurotrauma is a common cause of traumatic brain injury (TBI) and the "signature injury" in recent conflicts in Iraq and Afghanistan. Blast neurotrauma is complex and its mechanisms are not fully understood, especially for primary blast injury that is the direct result of an overpressure wave (Cernak et al., 1999; Ling et al., 2009; Rosenfeld et al., 2013). Most cases of blast neurotrauma are mild (concussion) and are not associated with gross damage to brain parenchyma (Rosenfeld et al., 2013; Chapman and Diaz-Arrastia, 2014; Bell et al., 2009).

We have recently developed and characterized a mouse model of primary blast neurotrauma using a specially designed shock-tube (Cernak et al., 2011; Koliatsos et al., 2011). This model generates neurological morbidity and mortality depending on shock-wave intensity (Cernak et al., 2011; Koliatsos et al., 2011). Under mild–moderate exposure conditions, subjects show traumatic axonal injury (TAI) in select tracts, especially optic and corticospinal, and develop mild cognitive/behavioral impairments (Koliatsos et al., 2011). One notable finding is that torso shielding significantly reduces TAI and mitigates behavioral defects, but head shielding does not offer any protection (Cernak et al., 2011; Koliatsos et al., 2011).

Neuroinflammation occurs frequently after blunt TBI, including concussion, and may contribute to secondary injury (Kumar and Loane, 2012; Morganti-Kossmann et al., 2002). Much less is known about

Abbreviations: CV, cresyl violet; DAB, 3,3′-diaminobenzidine; DAPI, 4′,6-diamidino-2-phenylindole; IHC, immunohistochemistry; IL-1ra, IL-1 receptor antagonist; NGF, nerve growth factor; ROI, regions of interest; TAI, traumatic axonal injury; TBI, traumatic brain injury; TGFβ, transforming growth factor β.

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neuroinflammation in blast neurotrauma. Neuroinflammation is featured by activation of microglia, i.e. mesodermally derived immunocompetent cells that exist in a resting state until they sense an injury signal and then become activated. A component of this activation is the formation of cells with classical macrophage properties that may further damage neural parenchyma (Morganti-Kossmann et al., 2002; Finnie, 2013; Acosta et al., 2013). Blocking microglial activation with minocycline may ameliorate neuropathology and functional or behavioral impairments in rodents subjected to TBI (Siopi et al., 2012; Homsi et al., 2010). Activated microglia can persist for long periods of time, a pattern indicating chronic neuroinflammation that may further damage the brain (Gentleman et al., 2004; Johnson et al., 2013).

In the present study, we investigated the temporal dynamics of neuroinflammation in the brain of mice exposed to mild–moderate blast in our established shock-tube model (Cernak et al., 2011; Koliatsos et al., 2011). Neuroinflammation was assessed by the presence of reactive microglia in injured brain tissues after mild–moderate blast, with and without torso protection. Although reactive astrocytes are part of the neuroinflammatory response to injury, these cells play complex roles that are difficult to dissect within the scope of this study (Ridet et al., 1997; Sofroniew, 2005; Anderson et al., 2014; Brenner, 2014; Pekny and Pekna, 2014; Pekny et al., 2014). The optic tract, one of the primary sites of TAI after blast (Koliatsos et al., 2011) was used here for purpose of quantitative assessment. Our findings are consistent with the view that neuroinflammation in primary blast neurotrauma tends to resolve one month after injury and confirm our previous findings that torso protection prevents key aspects of blast neurotrauma. These findings may assist the development of imaging strategies to monitor progression or resolution of primary blast neurotrauma and strategies to prevent secondary brain injury after blast with optimal armor protection or treat blast-induced neuroinflammation.

2. Materials and methods

2.1. Experimental subjects and shock tube set-up

The subjects of these experiments were 7–8 week old male C57BL6/J mice (Charles River Laboratories, Wilmington, MA). All animal care, operative and post-operative procedures were approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions.

Animals were subjected to blast neurotrauma via shockwave generated in a multi-chamber shock tube as described (Cernak et al., 2011; Koliatsos et al., 2011). Briefly, subjects were anesthetized with a gas mixture (isoflurane:nitrous oxide:oxygen 1:66:33) and placed in a multi-chamber shock tube consisting of two interlocking pipe compartments (a driver and a driven compartment) separated by an intervening Kapton-polyethylene diaphragm (Cernak et al., 2011). Anesthetized animals were fixed on a wire-mesh holder by tethering the four limps with cotton threads and the holder was positioned at the open end of the shock tube facing the shockwave front. To minimize movement of head, the upper frontal teeth were also tethered on the mesh by a cotton thread encircling the snout. Shockwave was generated by release of compressed helium in the driver compartment. Shockwave was adjusted to generate a membrane rupture pressure of 30 psig corresponding to mild–moderate blast injury (Cernak et al., 2011; Koliatsos et al., 2011).

To evaluate the role of body shielding in neuropathologies associated with blast injury, one group of subjects was fitted with a Plexiglas shield covering the chest and abdomen as described (Cernak et al., 2011; Koliatsos et al., 2011). Control mice were given sham lesions as previously described (Cernak et al., 2011; Koliatsos et al., 2011). These subjects were mounted to the side of the shock tube, thus they were exposed to the sound but not the shockwave itself.

Animals were allowed to survive for 1, 3, 7, 14 and 30 days (average $n = 5$ for each time point). Injured animals with torso protection were only allowed to survive for 7 days. All animals were euthanized with perfusion fixation and tissues were processed as described in next section.

2.2. Histology, general histochemistry and immunohistochemistry

At the appropriate times, animals were perfused with 4% freshly depolymerized, neutral-buffered paraformaldehyde. Brains were removed from skulls and immersed in the same fixative overnight at 4 °C. Tissues were cryoprotected and stored at −80 °C for further processing. Coronal brain sections (40 μm) were prepared in series for cresyl violet (CV), immunohistochemistry (IHC) and dual-label fluorescent IHC for markers of neuroinflammatory cells (the universal microglial marker IBA1 and the scavenger receptor/lysosomal membrane protein CD68 as a macrophage marker).

Immunoperoxidase-DAB based IHC was performed as previously described (Koliatsos et al., 2011). Briefly, sections were first incubated in the primary antibody overnight at 4 °C and then in biotin-conjugated secondary antibody (1:200; Jackson ImmunoResearch, West Grove, PA). Then sections were incubated in a solution of avidin and biotinylated HRP complex (VECTASTAIN Elite ABC Kit, VECTOR laboratories Inc., Burlingame, CA), then developed in 3,3′-diaminobenzidine (DAB). Some sections were counter-stained with CV. For fluorescent IHC, sections were incubated in secondary antibodies conjugated with Cy3 or Cy2 (1:200; Jackson ImmunoResearch, West Grove, PA) for 2–4 h at room temperature and then counterstained with the fluorescent DNA dye 4′,6-diamidino-2-phenylindole (DAPI) and coverslipped with DPX mounting media. Primary antibodies included: rat anti-CD68 (1:500, ABD Serotec, Raleigh, NC), rabbit anti-IBA-1 (1:500; Dako, Carpinteria, CA), mouse anti-KV1.3 voltage-gated potassium channel 1.3 (Kv1.3) (1:1000, NeuroMab Davis, CA). The latter epitope has been associated with presumably neurotoxic small ramified microglia (Yamada and Jinno, 2013). In the present study, no distinct above-background Kv1.3 (+) immunoreactivity was found in the optic tract 14 and 30 days post-injury using the same antibody as Yamada and Jino and no further comment will be provided in the Results.

Labeled sections were studied with a Zeiss Axiophot microscope and images were captured with a Spot RT Slider digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI). Confocal microscopy images were captured with pinhole set at 0.8 μm. Three-dimensional reconstruction by Z-stack scanning through regions of interest (ROI) was acquired with LSM software. Adobe Photoshop 7.0 software (Adobe Systems, San Jose, CA) was used for image processing.

2.3. Microglial profile counts in optic tract

Numbers of activated microglial profiles were counted in serial, systematically and randomly sampled coronal sections based on the optical fractionator concept as described (Yan et al., 2007). Counts were performed with an Axioplan microscope (Zeiss, www.zeiss.de) equipped with a motorized-stage and Stereo Investigator software version 10.01 (MicroBrightField, www.mbfbioscience.com). Every 24th of serial sections through the optic tract (from optic chiasm to cerebral peduncle) was subjected to stereological analysis. Contours were outlined at 5× and cells were counted using a 40× objective by a blinded investigator. A 125 × 125 μm counting frame was used with a 125 μm × 125 μm grid in the outlined area. Counting depth (optical dissector height) was 8–13 μm depending on average section thickness. A guard volume of 1.0 μm was used to avoid sectioning artifacts such as lost caps and uneven section surfaces. Density of microglia profiles was calculated by dividing estimated counts of profiles generated automatically from software with the estimated volume of the optic tract.

For CD68 (+) microglia, single cells and clusters of cells were counted separately. For IBA-1 (+) microglia, because of some difficulties in differentiating among various types of activated IBA-1 (+) microglial profiles, e.g. hypertrophied versus bushy/amoeboid and bushy/amoeboid