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Acute inflammatory response in rodent brain and blood following a blast induced traumatic brain injury

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Introduction

The predominant cause of neurotrauma among the military population is exposure to a blast wave. The shockwave produced by an explosive device can travel through the brain causing mild brain damage without any visible signs of injury. There has been a lot of interest in the role blast exposure plays in mild traumatic brain injury (TBI) and how this may link to the clinical features of the injury. TBI is a very complex disorder that is characterized by primary and secondary injury mechanisms (Loane and Faden, 2010). The primary injury mechanism is due to direct damage to the brain cells occurring at the time of the initial impact producing a series of biochemical processes which then result in the secondary brain injury. Secondary brain injury develops within minutes and can last up to months and includes a variety of processes among which is the initiation of inflammatory and immune responses (Frugier et al. 2010; Morganti-Kossman et al. 2007). The development of secondary injury events provides a window of opportunity for therapeutic intervention to prevent progressive tissue damage and loss of function after injury (Kumar and Loane, 2012; Loane and Faden, 2010).

Following neurotrauma, a complex cascade of cellular inflammatory response is initiated which could contribute to long term neurological deficits. The temporal profile of these inflammatory mediators released following brain damage could provide information about the injury severity. This study examined the central and systemic inflammatory response to TBI by comparing cytokine levels in rat brain and blood at various time points after a shockwave exposure.

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Methods

Animals: Male Sprague-Dawley rats (175-200g) were kept for at least one week before experiments commenced. The animals were housed in a room with a 12 h light-dark cycle (lights on at 0600h). Room temperature was maintained at 21 ± 2°C and relative humidity was 48 ± 3%. Food and water was available *ad libitum*. The experimental protocols were approved by the Defence Research and Development Canada Animal Care Committee prior to the use of any animals in this study.

Shockwave exposure: A custom-built blast simulator (approx. 30.5cm in diameter and 5.79m in length) was used for producing the simulated blast wave. Compressed helium and varying thickness of clear acetate sheets were employed to obtain the desired target pressure. The incident shockwave conditions which define the blast insult were characterized at minimum with respect to the peak (shock-front) amplitude, wave duration and impulse of the hydrostatic overpressure. Since the shock peak as measured is often affected by some degree of noise or irregular wave-front, peak overpressures were determined by using a curve-fit to the initial decay of the waveform. The anaesthetized animal was placed into a customized restraint which was inserted into the ABS section such that the animal's head was extended into the tube for a side-on, head only exposure. The head of the animal was located 4.3m downstream of the diaphragm and directly across from one of the pressure sensors mounted into the wall of the ABS so as to measure the incident static overpressure wave. Animals were exposed to a single shockwave with an intensity of 141 ± 1.3 kPa (20.5 ± 0.2 psi). After the shockwave exposure, the animal was removed from the shock tube and restraint. Sham control animals were anaesthetized, placed in the restraint and inserted into the blast simulator but they were not exposed to the shockwave. The recovery time from the anaesthetic was recorded. Controls and shockwave exposed animals were sacrificed either at 3, 6, 24, or 48 hours following exposure (n=7 per time points). Serum and brain tissue samples were collected at the time of sacrifice. All samples were stored at -80 ºC until analyzed.

Tissue preparation: The tissue preparation was carried out as previously described by Hulse et al. (2004) with some modifications. Briefly, all tissue was allowed to thaw on ice and then placed in cell lysis buffer with protease inhibitors. The samples were homogenized and then incubated at 4 °C for 15 min. The samples were centrifuged at 4500 x g for 15 min at 4 °C and the supernatant collected and stored at -80°C. All tissue samples were diluted with the cell lysis buffer to a protein concentration of 500ug/ml. Cytokine measurements on blood and tissue samples were carried out as previously described by Sajja et al (2012).

Immunohistochemistry: At 7d after the shockwave exposure, rats were anaesthetized and transcardially perfused with 4% ultrapure methanol free formaldehyde. The brains were removed and post-fixed with 4% formaldehyde for 48hours. Fixed brains were then dehydrated with 30% sucrose before storing at -80 °C. Thirty micron coronal sections were stained with a primary antibody against microglia/macrophage specific protein (Iba-1 anti-body; Wako Chemicals USA INC; 1:200) overnight at 4 °C followed by an appropriate secondary fluorescent antibody. At the end of the staining process, the nuclei were counterstained with DAPI. Stained sections were then mounted onto slides which were visualized and scanned using a WaveFX

Scanning Disk Confocal microscope with a 40X oil immersion objective. The morphology of microglial cells were compared between controls and shockwave exposed groups.

Results

Figure 1 Generation of blast waves: By using special wave-tailoring techniques, the Suffield Blast Simulator is able to produce shock waveforms simulating those from free-field explosives. These highly reproducible waveforms are characterized by a single pulse waveform with a short positive duration and a negative phase.

Figure 2 Increase in cytokine and chemokine levels: An acute cytokine/chemokine response was observed in the brain and serum of animals following exposure to a single shockwave.

- 3-6h post-exposure: IL-1α, IL-1β and IL-6 significantly increased in hippocampal tissues of the shockwave exposed animals. Similarly, elevated serum levels of IL-1α and IL-6 but not IL-1β, were observed in the brain-injured group of animals at these time points. Within hours after shockwave exposure, serum levels of the chemokines increased significantly as compared to the controls. Both chemokines levels were detected in the brain after 6h and remained elevated up to 48h post-exposure.

- 24-48h post-exposure: Serum levels of IL-1β were elevated at 24h and 48h. The anti-inflammatory cytokine, IL-10 levels in brain tissue samples showed an increase at 24h and 48h while a delayed increase in serum levels was observed for this cytokine (48h).

- At none of the time points measured post-exposure were elevated levels of TNF-α observed in the brain or serum of the blast-injured animals when compared to controls.

Figure 3 Microglial activation: Seven days after a single exposure to a shockwave, microscopic images of the hippocampal CA1 region showed much more strongly Iba-1 stained cells as compared to controls. These results indicate blast exposure induces activation of microglia in the hippocampus.

Discussion

In this study, the brain injury induced by shockwave exposure resulted in an early response in specific cytokines within the brain and blood. Specifically, TBI caused very early activation of IL-1α and IL-6 in the hippocampus and MCP-1 and MIP-1α in serum. Interestingly, an increase in IL-1α levels was detected within the brain and serum before IL-1β. Luheshi and colleagues (2011) also found a significant increase in the expression of IL1-α but not IL-1β in the brain tissue of mice within several hours following ischemia. These findings indicate that IL1-α plays an important role in the early stages of inflammation following brain injury.

In human studies, elevated levels of MCP-1 were observed for up to ten days in patients' CSF after severe brain injury indicating that this chemokine plays a significant role in mediating post-traumatic secondary brain injury (Semple et al. 2010). In a recent study by Cho et al. (2013), early changes were noted in pro-inflammatory mediators (IFN-γ and MCP-1) and a delay in microglial activation in the brains of rats following blast exposure. An increased in MCP-1 levels and a delay in microglial activation were also observed in this study. The changes in chemokines levels detected shortly after the blast-induced head injury would suggest that they are involved in the early stages of inflammation as well as the delayed recruitment of microglial cells into the injured tissues. Elevated levels of IL-10 were not detected until 24h following primary blast injury to the head demonstrating that the anti-inflammatory cytokine is involved in a delayed phase of the inflammatory process.

The early increase in several of the inflammatory cytokines observed in the brain before blood would suggest there is no clear relationship between the central and systemic inflammatory response to the brain injury induced by shockwave exposure. The temporal pattern of these cytokines/chemokines following neurotrauma illustrates the important role these immunological factors play in mediating regional damage. Determining the cytokine profile induced by brain injury could provide insights into neuroprotective approaches to treating this type of injury.

Reference

Frugier, T., Morganti-Kossman, M.C., O'Reilly, D., and McLean, C.A. (2010). In Situ detection of inflammatory mediators in post-mortem human brain tissue after traumatic injury. J. Neurotrauma. 27:497-507.

Hulse, R., Kunkler, P., Fedynyshyn, J., and Kraig, R. (2004). Optimization of multiplexed bead-based cytokine immunoassays for rat serum and brain tissue. J. Neurosci Methods 136: 87-98.

Kumar, A., and Loane, D.J. (2012). Neuroinflammation after traumatic brain injury: Opportunities for therapeutic intervention. Brain, behaviour and Immunity 26: 1191-1201.

Loane, D. J., and Faden A.I. (2010). Neuroprotection for traumatic brain injuries: Challenges and emerging therapeutic strategies. Trends in Pharmacological Sciences. 31: 596-604.

Luheshi, N.M., Kovacs, KJ., Lopez-Castejon, G., Brough, D., and Denes, A. (2011). Interleukin-1α expression precedes IL-β after ischemic brain injury and is localised to areas of focal neuronal loss and penumbral tissues. J. Neuroinflammation, 8:186.

Morganti-Kossman, M.C., Satgunaseelan, L., Bye, N., Nguyen P., and Kossman, T. (2007). Modulation of immune response by head injury. Injury 38:1392-1400.

Sajja, V.S., Tenn, C., Mclaws, L.J., and Vandevord, P.J. (2012). A temporal evaluation of cytokines in rats after blast exposure. Biomedical Sciences and Instrumentation, 48: 374-379.

Semple, B.D., Bye, N., Rancan, M., Ziebell, J.M., and Morganti-Kossmann, M.C. (2010). Role of CCL2 (MCP-1) in traumatic brain injury (TBI): Evidence from severe TBI patients and CCL2-/-mice. Journals of Cerebral Blood Flow and Metabolism, 30:769-782.

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Figures

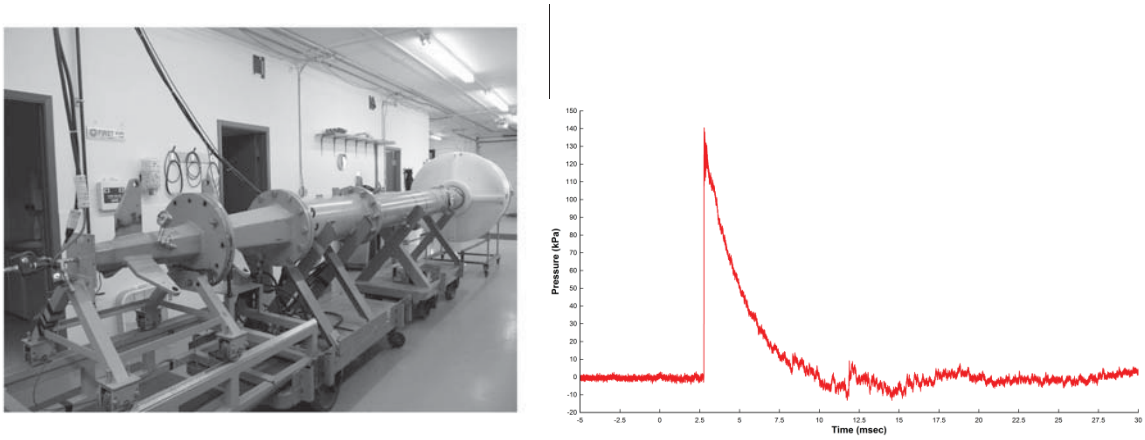

Figure 1.(a) DRDC Suffield Research Centre Blast Simulator. (b) Representative of a pressure-time history trace for the incident shockwave.

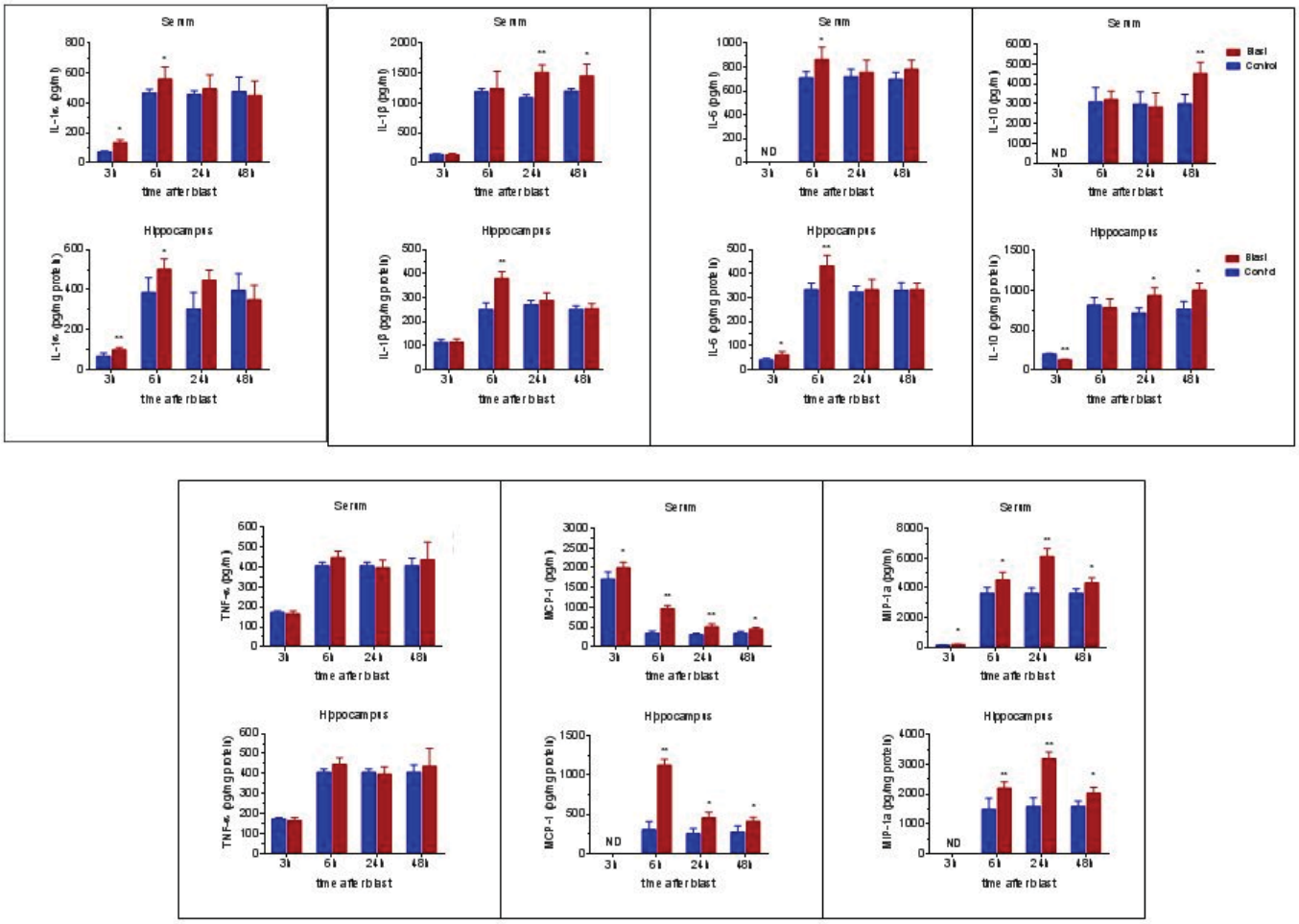

Figure 2. The expression of cytokines in serum and brain tissue at various time points following a single shockwave exposure of 141 kPa . Data represent means ± SD (n=7/group) * p <0.05 vs sham controls; ** p<0.01 vs sham controls.

Control Blast

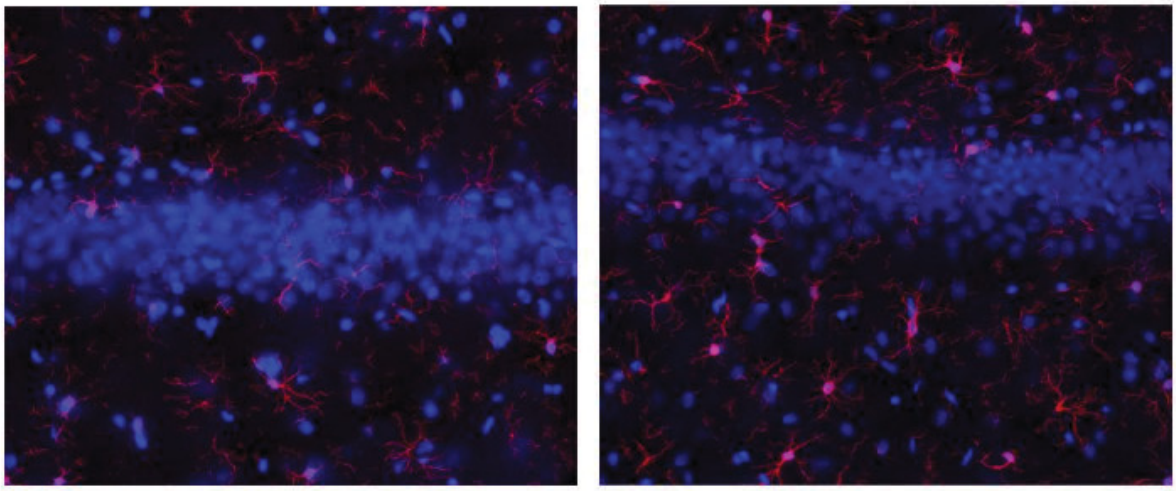

Figure 3 Representative confocal microscopic images of the CA1 region of the hippocampus of control (left) and shockwave exposed (right) animals. Hippocampal cells were stained with Iba-1 (red) and DAPI (blue). Magnification = 400x.