Blast Overpressure Induced Axonal Injury And Glial Reactivity Changes In Spinal Cord

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Introduction: Blast induced neurotrauma (BINT) is the signature wound of soldiers coming out of the military conflicts in Iraq and Afghanistan. Much of the ongoing research focus has been directed at understanding the changes in the brain1-3 with knowledge related to changes in the spinal cord remaining unresolved. Especially important is the knowledge related to secondary injury changes in the spinal cord following blast overpressure as they may be related to altered sensory changes often reported by veterans4. In fact, a high prevalence of chronic pain, particularly in the back (58%) and head (55%) in some veterans has also been reported5. Whether blast overpressure induces injury changes in the spinal cord and if these changes contribute to acute or chronic sensory changes is a fundamental question that needs to be addressed. We hypothesize that blast overpressure induces microscopic changes in spinal cord in the form of axonal injury (secondary axotomy) and glial proliferation. We further postulate that these axonal injury and glial reactivity changes show distinct spatial and temporal patterns. Accordingly, the purpose of this investigation is to assess axonal injury and glial reactivity in cervical thoracic and lumbar spinal cord in rats subjected to a single blast overpressure (22 psi) exposure at various post blast survival periods.

Methods: Anesthetized (isoflurane (3%) and 0.6 L/min oxygen) male Sprague Dawley rats (250-300 grams, Harlan Laboratories, Indianapolis, IN) without any chest protection were subjected to a dynamic overpressure in the range of 22 psi by a custom-built shock tube (12” diameter, 6 m shock-producing tube attached to 1 m exposure chamber (ORA Inc. Fredericksburg, VA; Figure 1A and 1B). Sham rats were subjected to all the procedures but not to blast overpressure exposure. The exposure pressure was determined by three sensors placed within the tube (2.4 m apart) and one placed on the platform holding the rat. Pressure measurements (Figure 1C) were collected at 250 kHz using a Dash 8HF data acquisition system (Astro-Med, Inc, West Warwick, RI). All rats were allowed to survive for 7 days. Axonal injury was studied by Gallyas silver staining and Neurofilament light (NF-L) chain immunohistochemistry (IHC). Astrocytic and microglial proliferation was studied by Glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) IHC respectively. The extent of astrocytic and microglial proliferation was quantified by counting their number from respective images taken from representative locations of cervical and lumbar spinal cord sections by using cell counter tool in ImageJ.

Results: Axonal injury: Preliminary studies in cervical spinal cord sections harvested at 7 days post blast revealed axonal injury in the form of axons appearing as beaded pearls or excessively wrinkled2 along with axonal debris (Figure 2B) by silver staining. Similarly, Neurofilament light chain (NF-L) immunohistochemistry (IHC) also revealed axonal injury in the form of axons with disrupted membranes, axonal swellings and retraction balls in large caliber axons (n=4; Figure 3A). No such changes were observed in sections from sham rats (n=3; Figure 2C).

Astrocytic proliferation:
7 days after blast exposure (n=5), significantly high GFAP immunoreactive profiles were observed in cervical and lumbar spinal cord sections compared to corresponding sections from sham (n=3; p<0.0; Figure 3).

Microglial proliferation:
7 days after exposure (single insult of 22 psi, n=5), significantly high Iba1 reactive microglial profiles were observed in sections from both cervical and lumbar spinal cord compared to corresponding sections from sham exposure (n=3; p<0.0; Figure 4).

Discussion: The results of this preliminary investigation support the presence of axonal and glial reactivity changes at 7 days after exposure to a single blast overpressure of 22 psi. These results also support the presence of extensive glial reactivity at various levels of spinal cord. Future studies are aimed at understanding axonal injury changes at cervical thoracic and lumbar spinal cord as well dorsal roots at the same levels and at 6 hours, 24 hours, 72 hours and 7 days. Future studies are also directed at studying the glial proliferation changes as well as inflammatory mediators (substance P, interleukin 1 beta and tumor necrosis factor) at the same time points from cervical, thoracic and lumbar spinal cord.

Significance: Taken together these studies will enable better a better understanding of the temporal progression of neuropathological sequelae of blast overpressure in an animal model especially those related to secondary injury changes at various spinal cord levels and dorsal roots. These may also offer the neurological basis of sensory changes following blast overpressure.

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Figure 1A shows a shock tube that will simulate a free-field shock wave. 1B shows the placement of a rat without chest protection. 1C shows driver pressure profile (blue) and rat as detected by a pencil (red) indicating the generation of a free-field pressure wave.

Figure 2 shows axonal injury changes by NF-L IHC in blast and sham sections. Also shown are axonal injury changes as revealed by silver staining.
Figure 3: A single insult of 22 psi blast overpressure induced significant (*) astrogial proliferation in cervical and lumbar spinal cord at 7 days.
Figure 4: A single insult of 22 psi blast overpressure induced significant (*) microglial proliferation in cervical and lumbar spinal cord at 7 days.

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