ELUCIDATING THE ROLE OF THE NEUROVASCULAR UNIT AND RELATED STRUCTURAL AND FUNCTIONAL NEUROLOGICAL SEQUELAE AFTER EXPOSURE TO BLAST

Usmah Kawoos[1,2], S.T. Ahlers[1], J.R Stone[3] and R. Abutarboush[1,2]

[1] The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD; [2] Naval Medical Research Command, Silver Spring, MD; [3] University of Virginia School of Medicine, Charlottesville, VA

Introduction

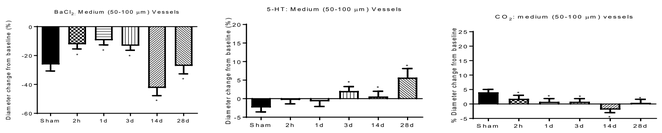
- Repeated exposures to blast in training and operations are a prevalent concern among military personnel.
- In the recent years there has been an increasing body of evidence suggestive of the linkage between exposures to low-intensity blasts and long-term neurological sequelae.
- Our group and other researchers have shown that blast overpressure (BOP) may result in cerebrovascular impairment including cerebral vasospasm, with possible subsequent neuropathology.
- Blast-related acute and chronic alterations in the neurovascular unit (NVU-neurons, astrocytes, vasculature (endothelial and vascular mural cells), the vasomotor apparatus (smooth muscle cells and pericytes), and microglia) were reported, which may have a role in the evolution of neurological sequelae after blast exposure(s).
- The key findings from our work on preclinical and human studies are presented here.

Key Findings

- Preclinical studies characterized the effects of BOP on cerebrovasculature and the NVU.
- Functional assessment of cerebrovascular alterations after exposure to a single ~130 kPa (18.8 psi) BOP was done by measuring pial arteriolar tone in response to stimulation by mechanistically varied vasoactive mediators; permeability of blood-brain barrier; brain tissue oxygenation; and cerebral blood flow.

Functional Alterations

Vascular Reactivity

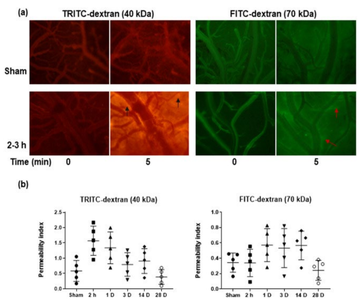


Barium chloride ($BaCl_2$, vasoconstrictor)
BOP induced a time-dependent increase in contractility

Serotonin (5-HT, vasoconstrictor)
BOP reverses the response to 5-HT over time

Hypercapnia (CO_2, vasodilator)
BOP impairs vasodilation

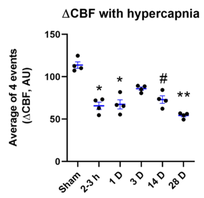
Blood-Brain Barrier (BBB)



Blast damages BBB
Extravasation of fluorescent probes from pial vessels seen after BOP exposure.

Increased BBB permeability within 2-24 hours after BOP correlates with the reduction in tight junction proteins (ZO1, claudin5, and occludin).

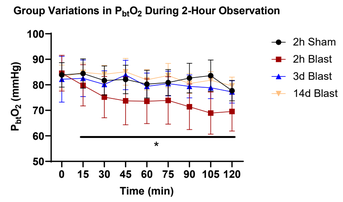
Cerebral Blood Flow (CBF)



Blast alters CBF response to hypercapnia
Blast-related enduring CBF changes in response to CO_2.

Changes in CBF after blast exposure may not be evident until challenged.

Brain Tissue Oxygenation ($P_{bt}O_2$)

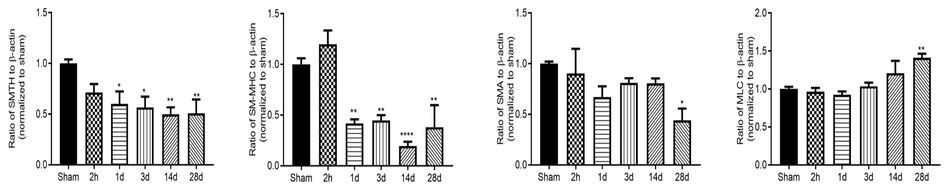


Blast reduces brain oxygenation acutely
Significant reduction (~30%) in $P_{bt}O_2$ within the first few hours after exposure.

Key Findings

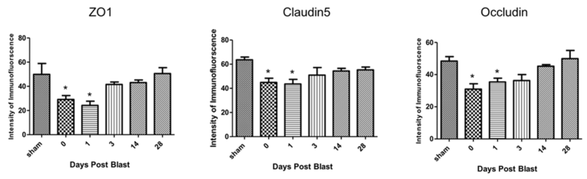
Structural Alterations

Smooth Muscle



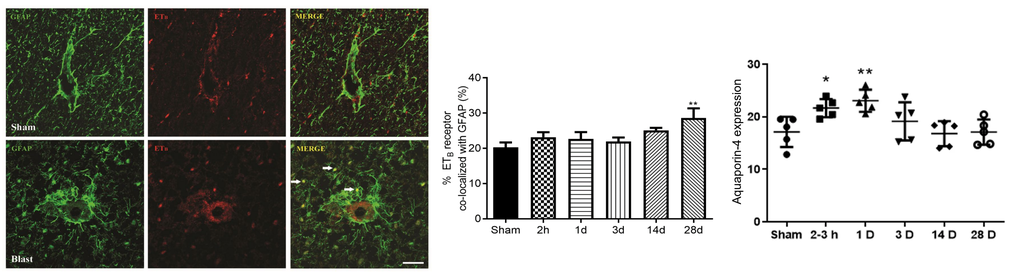
Blast induces changes in smooth muscle contractile proteins
Loss of contractile smooth muscle proteins. Delayed increase in myosin light chain 28 days after blast.
SMTH: smoothelin, SM-MHC: smooth muscle myosin heavy chain, SMA: smooth muscle actin, MLC: myosin light chain

BBB Tight Junction (TJ) Proteins



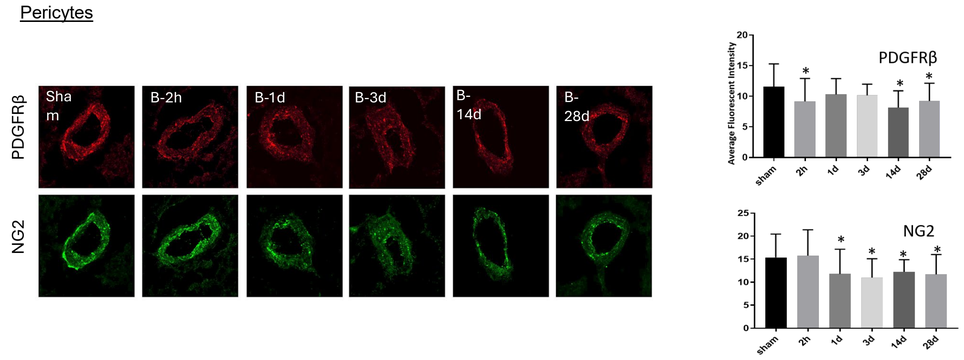
Blast-related reduction in tight junction proteins
Time course of reduction in TJ proteins maps on the increase in BBB permeability.

Astrocytes



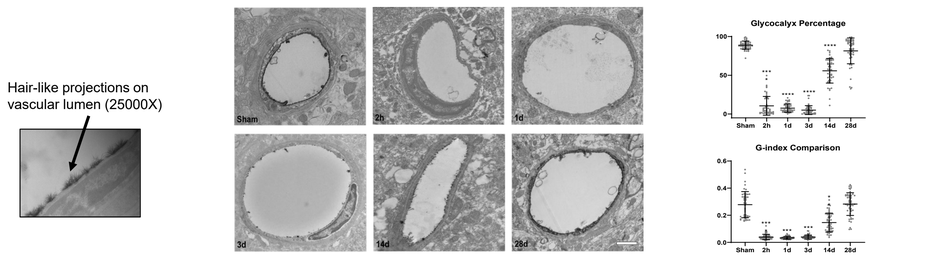
Blast is associated with changes in astrocytic proteins
ET_B (endothelin receptor B) co-localization with astrocytes increased 30% 28 days after blast exposure.
Significant increase in the expression of AQP-4 (aquaporin-4, a water channel protein in astrocytic endfeet) acutely after exposure.

Pericytes



Blast-related reduction in markers of pericytes
Gradual reduction in pericyte markers (platelet derived growth factor-receptor-β, PDGFR- β and neural/glial antigen-2, NG2) over the 28-day time course after blast.

Glycocalyx



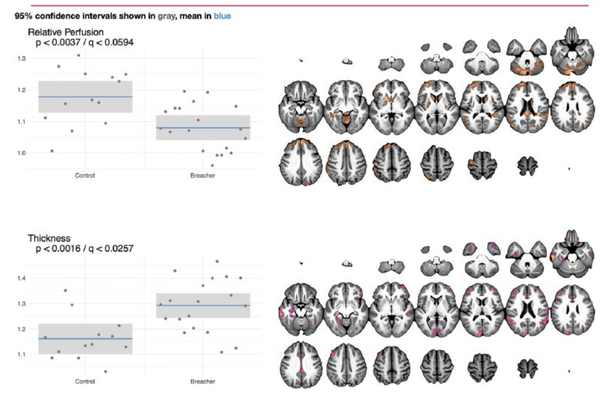
Blast cleaves endothelial glycocalyx
Time-dependent shearing of the cerebrovascular endothelial glycocalyx is evident 2 hours after exposure and lasts up to 3 days.
Endothelial glycocalyx recovers by 28 days as is associated with a decrease in hyaluronic acid during the period when regeneration is observed.

In a repeated low-intensity blast model a pervasive reduction of cerebral glycocalyx is seen in different brain regions (hippocampus, amygdala, cortex, striatum, corpus callosum). Administration of hyaluronidase reduces the loss of glycocalyx from repetitive low-intensity blast exposure. (Data not shown)

Key Findings

- While evidence continues to emerge concerning the specific neurobiological underpinnings of repeated low-level blast exposure in military populations, it is becoming increasingly clear that the cerebral vasculature is selectively vulnerable to BOP exposure. Our studies with military personnel using imaging methods have focused on exploring the neurological correlates of blast exposures.
- We have shown that repetitive exposure to BOP over a career is associated with distinct neurological changes including alterations in cortical thickness and relative perfusion.
- Recent work from our group exploring the cumulative neurological effects due blast exposure from artillery fire demonstrates alterations in cerebral blood flow in artillery exposed personnel within the lateral occipital and superior parietal regions. The degree of cerebral blood flow alteration was highly correlated with career exposure to artillery rounds.

Cortical Thickness and Relative Perfusion



Repeated blast exposures affect cortical thickness and relative perfusion
Imaging data from experienced breachers shows a significant increase in cortical thickness. A reduction in relative perfusion is also seen. Given that NVU plays a critical role in cerebrovascular autoregulation, altered cerebral perfusion implicates the disruption of NVU as a contributing driver of perfusion changes.

Conclusions and Future work

- Collectively, the preclinical data shows that exposure to a single high intensity blast results in delayed and prolonged alterations in cerebrovascular reactivity, blood-brain barrier, and cerebral blood flow that are associated with both acute and chronic changes in the microarchitecture of the vessel wall, pericytes, and astrocytes. Repetitive exposures to low intensity blasts are also associated with altered cerebrovascular function and structure (data not fully presented). These changes may contribute to long-term pathologies involving dysfunction of the NVU.
- Our findings from studies with operational personnel (experienced breachers and artillery) further underscore the role of NVU in long-term neurological sequelae after repeated low-level blast exposures.
- These observations fueled a recently funded research program focusing on elucidating the involvement of NVU dysregulation in the long-term impairment of brain health in military and veteran populations exposed to repetitive BOP exposure in their career. The program will utilize multiple fluid-based, imaging-based, and physiological approaches with the potential to be translated towards the detection and mitigation of altered cerebrovascular components affecting vascular regulation of blood flow in the brains of military personnel exposed to repeated BOP. In addition, a pre-clinical animal model will be used to characterize the effects of repeated BOP on cerebrovascular function and assess a treatment strategy targeting the cerebrovasculature. In summary, assessing blast effects on the NVU is pivotal for understanding the injury mechanism and long-term neurological consequences, identifying biomarkers, developing therapeutic targets, and preventing chronic pathology.

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IACUC statement: The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Command Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research