Preclinical Studies for the Treatment of Blast-related Injuries

Functional and Structural Changes in Cerebral Vasculature Following Exposure to Blast

Explosion or blast is the most common cause of war injuries in Operation Enduring Freedom and Operation Iraqi Freedom (*Warden 2006*). Exposure to multiple low intensity blast events with or without overt concussion has an additive effect with long-term neurologic and other health consequences (*Ling et al. 2009*). Despite clinical indications of vascular insult and supporting experimental data in animals, there remains a paucity of information on specific structural and functional changes in the cerebral vascular space that occur after blast exposure. The studies being conducted at the Naval Medical Research Center (Silver Spring, Maryland) use an established rodent (Rattus norvegicus) model (*Abutarboush et al. 2013*) to assess the effects of a single exposure to blast overpressure (BOP) intensities on functional and structural changes in cerebral vasculature.

These studies used an established cranial window rodent model to assess the effects of a single exposure to varying BOP intensities on cerebral macro- and micro-vasculature for up to 90 days after exposure with an emphasis on identifying physiological underpinnings associated with cerebral vasospasm. Study techniques included functional indices of vascular function using intravital microscopy (IVM) to assess changes within the cerebrovascular responsiveness after exposure to BOP. Continued work includes IVM and immunohistochemistry in evaluating any changes in vasculature as well as Blood Brain Barrier (BBB). Alterations in BBB permeability are investigated in vivo and in vitro. Functional changes in BBB permeability were observed in vivo in real time using IVM. The structural changes in cerebral vasculature are being studied via immunohistochemistry- following identification of alterations in some of the structural components of BBB and vascular smooth muscle. The results indicate that there is a change in BBB permeability within 24 hours after BOP exposure. The blast-related changes in the structure of vascular smooth and the resulting remodeling of the vasculature may be responsible for changes in the reactivity of vessels to vasoactive mediators. Studies to assess structural effects are accomplished using electron microscopy coupled with immunostaining techniques to visualize changes to vascular endothelium; these specifically include the glycocalyx, assessment of tight junctions, perivascular edema, and changes in endothelin-1 within the vasculature. Experiments examining cerebrovascular responsiveness using IVM at two hours, one day, three days, 14 days, and 30 days after exposure to a single 37, 75, or 140 kilopascal explosive device are currently still in progress. To date, data has been gathered from 200 rats, which completes approximately 75 percent of the planned IVM experiments (Figures 1 and 2). Protocols for examination of the glycocalyx and BBB using immuno-electron microscopy have been refined to ensure consistent, high quality images.

Collaborators at the University of Virginia (Charlottesville, Virginia) are concomitantly examining vessels with magnetic resonance angiography and have completed optimization of their perfusion and BBB integrity sequences; initial data acquisition has recently begun. Data from this research was presented in July at the National Neurotrauma Symposium (*Abutarboush et al. 2017*).

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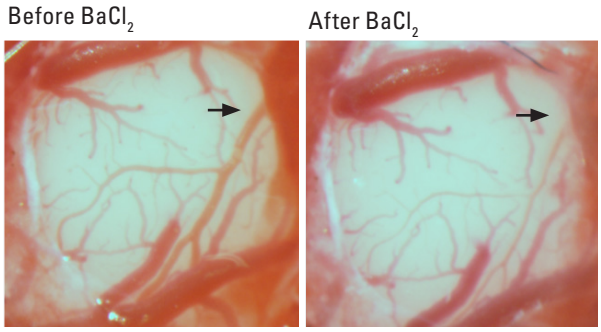

FIGURE 1: Intravital microscopy images of the pial microcirculation demonstrating the change in pial arteriolar diameter after the topical application of a 5 percent barium chloride (BaCl2) solution. Arrows point to constricted pial arterioles within an artiolar tree. BaCl2 was one of three substances used to assess vascular function after exposure to blast by probing the ability of arterioles to respond to different vasoactive mediators. (Figure used with permission from the authors)

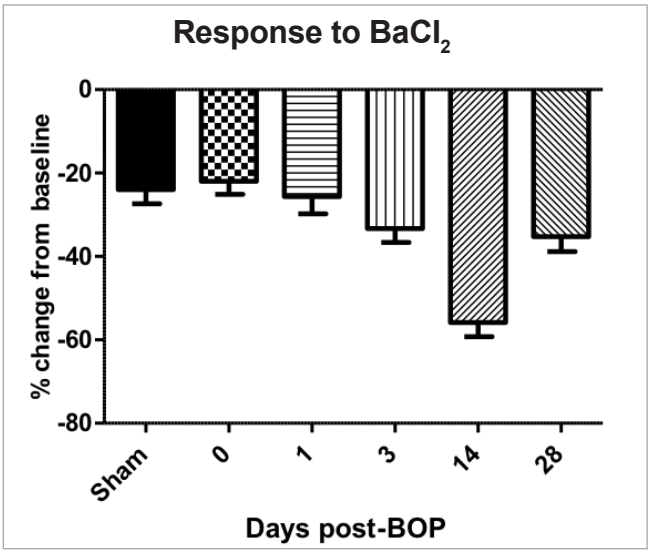

FIGURE 2: Exposure to a single 140 kilopascal blast causes a delayed change in the responsiveness to BaCl2. There is an increase in vascular contractibility in response to BaCl2 compared to sham non-blast animals that starts three days post-blast and peaks at 14 days ($p < 0.05$). (Figure used with permission from the authors)

REFERENCES:

Abutarboush, R., Mullah, H., Lashoff-Sullivan, M., Chen, Y., Arnaud, F., Kawoos, U., McCarron, R., and Ahlers, S. T. 2017. "Alterations in Cerebrovascular Responsiveness after Blast Correlate with Changes in Endothelial Function-Related Factors." J Neurotrauma 34 (13):A87-A87.

Abutarboush, R., Scultetus, A., Pappas, G., Arnaud, F., Auker, C., McCarron, R., and Moon-Massat, P. F. 2013. "Effects of N-Acetyl-L-Cysteine and Hyaluronic Acid on HBOC-201-Induced Systemic and Cerebral Vasoconstriction in the Rat." Curr Drug Discov Technol 10 (4):315-24.

Ling, G., Bandak, F., Armonda, R., Grant, G., and Ecklund, J. 2009. "Explosive Blast Neurotrauma." J Neurotrauma 26 (6):815-25. doi: 10.1089/neu.2007.0484.

Warden, D. 2006. "Military TBI During the Iraq and Afghanistan Wars." J Head Trauma Rehabil 21 (5):398-402.