Functional Motor Deficits and Brain Stem Damage Resulting From a Focused Blast Overpressure Model of Traumatic Brain Injury

ATACCC 2009

J. Travis Parsons, PhD[1,4], Jiepei Zhu, MD, PhD[1,4], Cameron R. Smith, PhD[1,4], Dong Sun, PhD[3] Bruce D. Spiess, MD[1,2,4]

Departments of Anesthesiology[1], Emergency Medicine[2], Neurosurgery[3] and the Virginia Commonwealth University Reanimation Engineering Shock Center (VCURES)[4], Virginia Commonwealth University, Richmond, Virginia, USA

BACKGROUND

Traumatic brain injury (TBI) due to blast exposure is a major concern for troops in Afghanistan and Iraq. Soldiers injured are surviving in greater numbers than in past conflicts and while medical techniques can improve survival, some soldiers have long-term neurological deficits. With the current conflicts, second echelon treatment centers report 88% of the injuries as a result of blast. Little is known about the brain injuries sustained by those surviving blast exposure, and even less is known regarding the consequence of exposure to multiple blasts. Understanding the relationship between primary blast wave exposure and brain injury is critically important for finding promising treatment options.

VCURES designed a blast wave generator that is a modification of that originally described by J. H. Jaffin et. al. Unlike models that depend on explosives, this device can be safely operated in a lab environment in the presence of equipment unable to be used in the field and in the presence of explosives. The compressed air generator yields a pressure wave comparable to a chemical explosive such as TNT or C4. The unit has a focused blast nozzle and mimics a simple free field blast wave. Thus, the effect of a controlled single pressure pulse or several controlled pressure pulses can be studied as opposed to a complex blast wave with multiple secondary pressure waves of variable intensity.

METHODS

Blast Induced Traumatic Brain Injury

- Male Sprague-Dawley rats (375-425g) were anesthetized with 4% isoflurane until unconscious and maintained at 2% isoflurane for the duration of the procedure
- Rats were then placed in the prone position, placed on the blast platform, and positioned such that the center of the blast nozzle was aimed at the top of the rat head centered directly between the ears
- The distance between the blast nozzle and the top of the head was 7 cm.
- Following blast exposure, animals were subjected to behavioral studies followed by brain processing for histological assessment

Behavioral Assessments

Beam Walk

- Rats were placed on one end of beam, subjected to a bright light and loud noise, and the amount of time required to cross the beam and reach safe dark box was recorded
- Rats were trained two days prior to blast and must finish beam walk in less than 5 sec for 3 consecutive trials
- Rats were pre-assessed one day prior to blast, three trials each rat
- Rats were then assessed at 24, 48, and 72 hours post blast exposure

Beam Balance

- Rats were placed on a narrow beam and were required to maintain balance on beam for up to 60 sec
- Rats were also scored as follows: 1 – balance with steady posture, 2 – fairly steady some shakiness, 3 – little movement shaky posture, 4 – balances greater than 10 sec but less than 60 sec, 5 – balances less than 10 sec, 6 – makes no attempt to balance
- Rats were trained two days prior to blast and must balance for 60 sec with a score of 2 or less for 3 consecutive trials
- Rats were pre-assessed one day prior to blast, three trials each rat
- Rats were then assessed at 24, 48, and 72 hours post blast exposure

Histological Assessments

- 24 hrs following blast, rats were deeply anesthetized, perfused with 4% paraformaldehyde, brains removed and sliced at 50 µm with a vibratome for subsequent staining
- Hematoxylene and Eosin Y (H&E) stain: slices were hydrated, incubated in Harris hematoxylene, rinsed, differentiated in acid alcohol, rinsed, stained with 1% eosin, rinsed, dehydrated, and placed in organic solvent before mounting
- β-Amyloid Precurser Protein (APP) stain: immunohistochemistry, primary anti-APP antibody 1:1000 incubated over night at 4° C, then with biotinylated secondary antibody 1:500 for 60 minutes, ABC kit (Avidin and Biotinylated horseradish peroxidase [HRP] Complex; vector) 1:1000 for 60 minutes, visualized with DAB (Diaminobenzidine).

Figure 1: Laboratory Equipment and Experimental Setup

Beam Balance Behavioral Assessment Apparatus | Beam Walk Behavioral Assessment Apparatus | Rat in position below high pressure, low volume blast nozzle

RESULTS

Figure 2: Contusions as a Result of Blast to the Brain and Ear

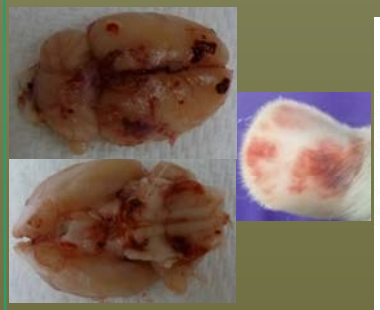
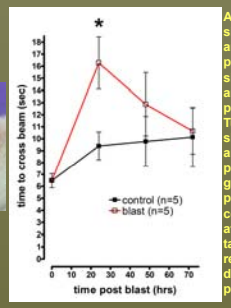
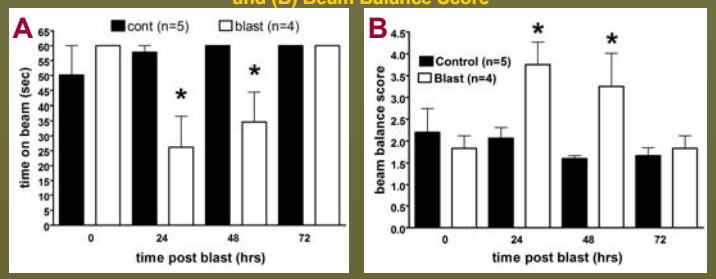


Figure 3: Control vs Blast Beam Walk Assessment



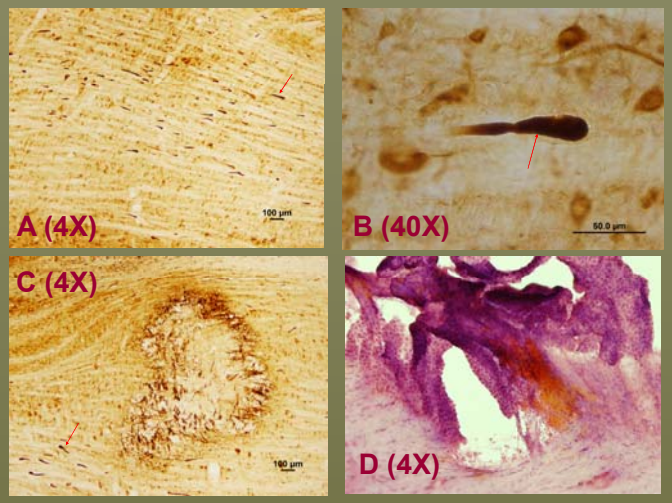
ANOVA reveals no significant difference among all control time points. ANOVA reveals significant difference among all blast time points (α=0.0003). Tukey post-hoc test showed 24 hr (p<0.001) and 48 hr (p<0.05) time points significantly greater than 0 hr time point. Comparing control and blast data at each time point, two-tailed unpaired t-test revealed a significant difference at 24 hrs post blast (*p=0.02)

Figure 4: Control vs Blast (A) Beam Balance Assessment and (B) Beam Balance Score



ANOVA reveals no significant difference among all control time points. (A) ANOVA reveals significant difference among all blast time points (α=0.01). Tukey post-hoc test showed 24 hr time point significantly different than 0 and 72 hr time points (p<0.05). Comparing control and blast data at each time point, two-tailed unpaired t-test revealed a significant difference at 24 and 48 hrs post blast (*p<0.03). (B) ANOVA reveals significant difference among all blast time points (α=0.04). Comparing control and blast data at each time point, two-tailed unpaired t-test revealed a significant difference at 24 and 48 hrs post blast (*p<0.05).

Figure 5: Histological Assessment of Brain Injury 24 Hrs Post Blast. APP Stain Showing axonal damage (A,B) and Bleeding (C) in the Brainstem. H&E Stain (D) Demonstrating Ventricle Bleeding



CONCLUSIONS

- Pressure in the blast nozzle at the time of detonation was 1825 ± 25 psi. VCURES researchers have recently obtained calibrated piezo electric crystal pressure transducer and oscilloscope to determine the pressure at the point of impact on the rat skull
- Pressure nozzle (6.3 x 2.4 cm) generates a focused, high pressure, low volume air blast
- Out of 7 animals that were blasted, 3 died giving a mortality rate of 42.9%
- Out of 7 animals that were blasted, 6 had significant seizure activity immediately post blast
- 24 hrs post blast, control animals beam walk response time was 57% (nearly twice as fast) lower than blast animals
- On the beam balance, blast animals could hold on only 45% and 58% (control animals held on twice as long) at 24 hrs and 48 hrs post blast, respectively, compared to control animals
- Beam balance scores of control animals were 55% and 48% at 24 hrs and 48 hrs post blast, respectively, compared to control animals
- This blast model produces significant contusion to the brain and ears as well as significant neuronal death and bleeding in the brainstem as well as bleeding in the cortex
- The data presented suggest that blast to the top of the head of the rat causes significant cell death and intra-brainstem bleeding that is associated with temporary loss of motor skill function
- It is highly probable that exposure to a second blast during the 24 to 48 hour window following the first blast could lead to more extensive and permanent neuronal dysfunction

Acknowledgements: Funded by Office of Naval Research Grants N000140810366 to BDS