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TITLE: Treatment of Traumatic Brain Injury by Localized Application of Sub-atmospheric Pressure to the Site of Cortical Impact

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14. ABSTRACT Each major war tends to have a 'signature injury', with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). The large gyrencephalic brain of swine is similar to humans, thus a swine model of a controlled cortical impact (CCI injury) treated by the controlled application of sub-atmospheric pressure was employed. Work completed in Year 2 showed that application of 100 mm Hg to the site of the CCI for 5 days was necessary to prevent a late increase in intracranial pressure (ICP). Four of the 10 animals treated with 100 mm Hg vacuum for 3 days showed an increase in intracranial pressure. Thus, application of 100 mm Hg sub-atmospheric pressure for five days was necessary to prevent a late increase in intracranial pressure.					
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Introduction

Each major war tends to have a ‘signature injury’, with traumatic brain injury (TBI) associated with the Iraq war (Operation Iraqi Freedom II and Operation Enduring Freedom) due to the high incidence of personnel injured by IED (improvised explosive devices). Our previous study in a rat model showed that Mechanical Tissue Resuscitation (MTR – the controlled application of vacuum) to the cerebral cortex following a controlled cortical impact (CCI) injury reduces brain edema and the extent of injury, modulates metabolites in injured neuronal tissues, preserves neuronal tissue, and improves functional recovery. The large gyrencephalic brain of swine is similar to humans, thus a swine model of CCI injury and MTR treatment was evaluated for future human clinical applications. Year 2 goals were to determine the length of time that the 100 mm Hg of applied sub-atmospheric pressure (determined in Year 1) was necessary to prevent late cell injury and death and late increase in intracranial pressure.

Body

Year 2 goals stated in the Statement of Work included determination of the length of time for application of sub-atmospheric pressure necessary to prevent cell death and injury due to secondary injury, and also to prevent a late increase in intracranial pressure. Animals were treated for 1, 3 or 5 days with application of 100 mm Hg vacuum to the site of the focal trauma (level of sub-atmospheric pressure determined in Year 1).

Thirty four (34) female domestic swine (22-33 kg) were procured. For MTR treatment, a sterile vacuum dressing was placed in the bony defect and 100 mm Hg was applied continuously for 1, 3 or 5 days. Intracranial pressure was monitored by telemetry. Five days post surgery, all animals were analyzed by MRI (GE Signa EchoSpeed 1.5-T scanner). Parameters analyzed included: MR sagittal T1 imaging; coronal T2 imaging; coronal MPGR (Multi-Planar Gradient Echo); Axial T2* Contrast Enhanced Perfusion (0.2 ml/Kg Magnevist contrast by power injection). All animals were euthanized 10 days post injury and perfused with 4% para-formaldehyde through the ascending aorta 8 days post-injury. The brain was removed and postfixed in the same fixative solution overnight at 4°C. After a PBS rinse, the brains were placed in 30% sucrose at 4°C before they were snap-frozen in O.C.T and stored at -80°C. Coronal sections of the injured area were cut into 20 µm thick sections using a cryostat, mounted, and kept frozen until use. Sections were collected every 0.5 mm through all injured area over a total distance of 2 cm. Histological staining and analysis is being completed.

All 10 animals in the 5 day treatment group survived (100% survival) with one suffering a seizure. Three of the six animals (50%) died prior to day 10 post injury. (Figure 1) The 3 day treatment was discontinued due to the high level of mortality. The 1 day treatment was not performed due to the high mortality rate in the 3 day treatment group.

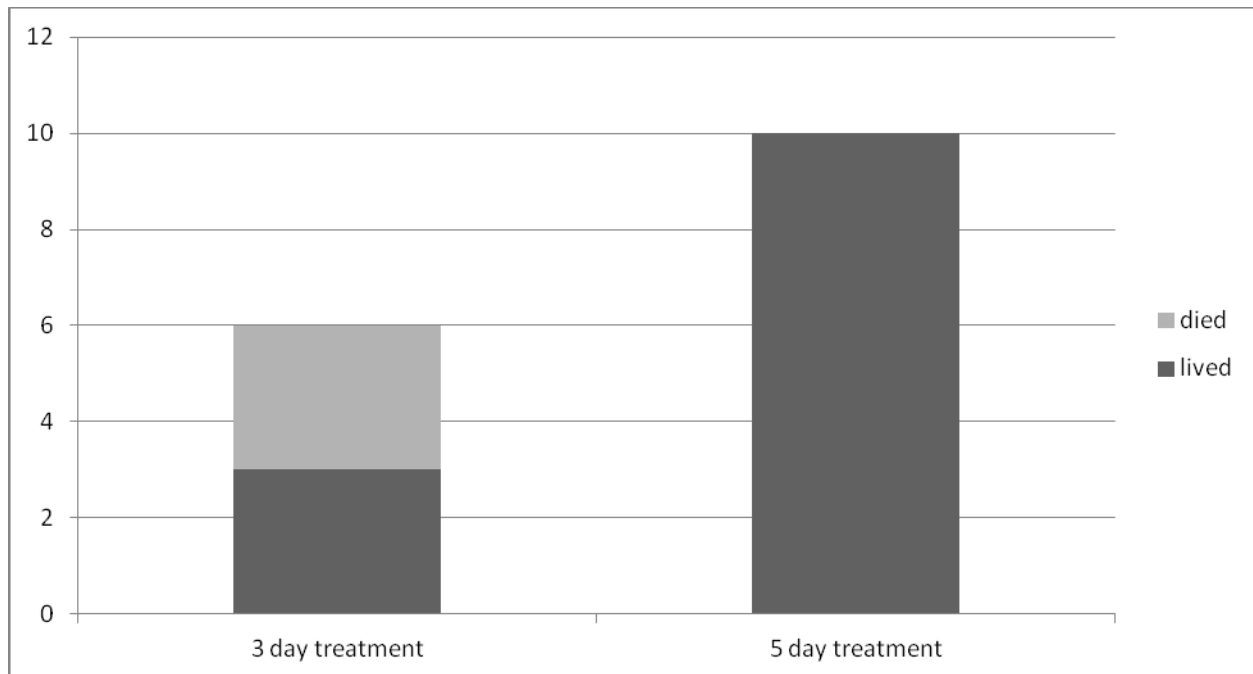


Figure 1. Bar graph showing survival rates for animals in either the 3 day treatment group (50% survival) or the 5 day treatment group (100% survival).

The volume of cerebral hemorrhage for animals treated for 5 days (146 ± 71 mm³ - SPS dressing and 166.2 ± 67 mm³ - WS dressing) was significantly less ($p < 0.2$) than for animals in then on-treated control group (564 ± 112 mm³). (Mean $\pm$ SEM) Figure 2.

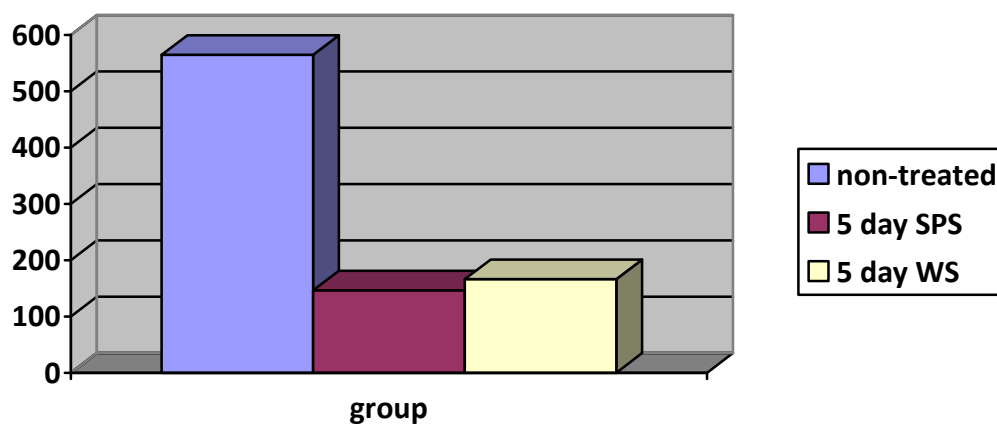


Figure 2. Hemorrhage volume at site of injury as measured from MRI. Treated groups had significantly less hemorrhage volume than non-treated group.

T2 weighted MRI's of the animals treated for 5 days showed a significant decrease in water content (swelling) in the area of injury. Also evident from the images is the herniation of the

brain through the craniotomy for the non-treated group. No herniation is evident for the animals treated for 5 days. Two different vacuum dressings were employed (labeled SPS and WS in Figure 3 below) with equal efficacy.

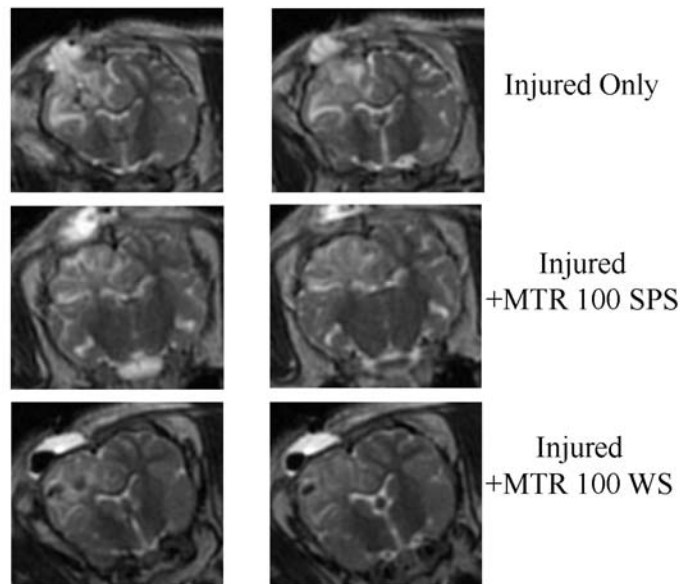


Figure 3. T2 weighted MRI of non-treated and 5 day treatment animals. Injured site is on left side of brain. Herniation of the brain through the craniotomy is visible in the non-treated image (top). Two different vacuum dressing were employed (SPS (middle) and WS (bottom)) with equal efficacy.

Key Research Accomplishments

- Determination that application of 100 mm Hg sub-atmospheric pressure for 5 days to the site of cortical injury is necessary to prevent a late increase in intracranial pressure. 100% (n=10) of the animals treated for 5 days survived (although 1 did suffer a seizure). 50% (3 of 6) of animals treated for 3 days died by day 10 post injury. Treatment for 1 day was not performed due to the high mortality of animals in the 3 day treatment group.

Reportable Outcomes

An abstract of the preliminary results of Year 2 was submitted, the abstract accepted, and the results were presented at the 27th Army Science Conference.

Morykwas M, Zheng Z, Bryant A, Argenta L: Mechanical Tissue Resuscitation Treatment Reduces Brain Tissue Volume and Intracerebral Hemorrhage in a Pig Traumatic Brain Injury Model. 27th Army Science Conference. Orlando, FL. Nov 29- Dec 2, 2010.

Conclusion

This portion of the study demonstrates that the use of mechanical tissue resuscitation (MTR) treatment of 100 mm Hg for 5 days is necessary to prevent late increases in intracranial pressure due to secondary cell death and injury. Animals treated for less than 5 days (3 days) exhibited a 50% mortality.

Year 3 studies have been initiated to determine the maximum length of time between the time of injury and successful application of vacuum to the site of the injury.

The expectation of rapid translation of the technique to humans is still anticipated with interest from industry for commercialization of the product and technique, although no formal licensing discussions have begun.

References

N.A.

Appendices

N.A.