Measurement of GFAP and CD68 in Brain Tissue after a 'Hit and Run' Injury

Rebecca Giese: Department of Chemistry, High Point University, High Point, NC 27262

Center for Translational Neuromedicine, University of Rochester, Rochester NY, 14617

Introduction

Each year, as many as 3.8 million mild head injuries occur from sporting activities in the United States. Additionally, more than 260,000 American have suffered mild traumatic brain injury since 2002. Consider a soccer player; every day whether in a game or at practice they are either hitting their head with the ball or falling to the ground. But how do these mild head injuries that are usually 'shaken off' affect the brain and the individual? Or a soldier on the war front that is hit with the back blast of an explosion. Both the soccer player and the soldier appear normal from a behavioral standpoint, but their brains actually may have undergone damage of unknown severity. The goal of this research was to study brain damage after the administration of a traumatic brain injury of varying degrees. This was done by observing the atrophy and glial cell activation in the brain tissue of mice. It was determined that brain damage is occurring at all injury levels- mild, moderate and severe.

Experimental

Animals and Injury Model:
For this research, mice were used as a study subject and the brain injury (mild, moderate, or severe) was administered using the 'Hit and Run' model.

Tissue Sampling and Preparation:
Brain tissue samples were taken randomly at the 3, 7, 14 and 28 day marks after the administered injury. Samples were sliced and stained for imaging. DAPI stains for the nucleus, GFAP indicates the activation of astrocytes, while CD68 indicates the activation of microglia.

Imaging and Analysis:
Images of the whole brain were taken with a fluorescence imaging microscope and were analyzed using Image J software. With this software, degrees of atrophy, and GFAP and CD68 expression were evaluated.
Images of neurons and astrocytes were taken with a confocal microscope and analyzed for cell count using Image J software. This provided information on cell death for varying levels of injury at differing time intervals. The number of astrocytes were also counted to determine the correlation between cell death and glial activation.

Data

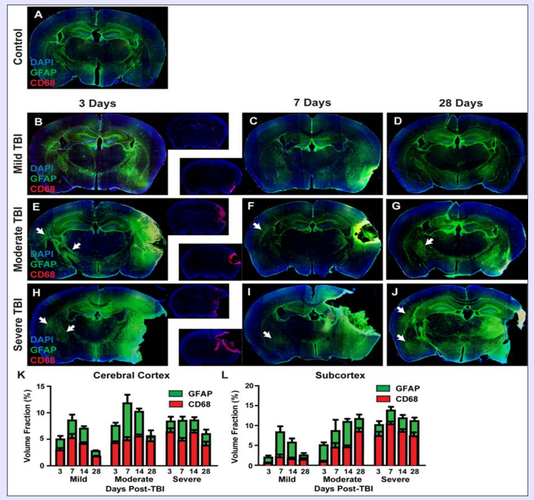

Figure 1: **Fluorescence Imaging**
Full brain images taken with the fluorescence imaging microscope showing DAPI, GFAP and CD68. (A) is the control image. (B), (C), (D) are images of brains with mild TBI at the 3, 7, and 28 day marks, respectively. (E),(F),(G) are images of moderate TBI at the 3, 7, and 28 day marks, respectively. (H), (I), (J) are images of severe TBI at the 3, 7, and 28 day marks, respectively. (K) represents measurements taken with Image J of GFAP and CD68 concentrations in the cerebral cortex, while (L) represents these concentrations in the sub cortex.

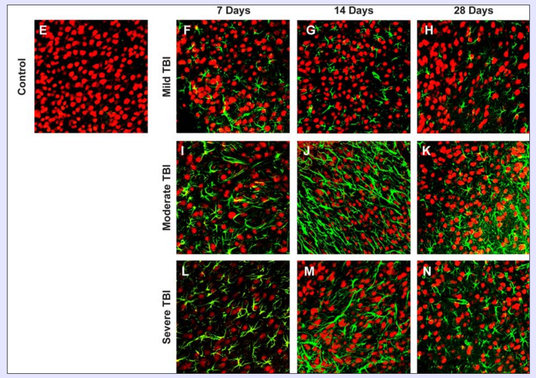

Figure 2: **Confocal Microscope Imaging**
Red indicates neuronal presence and green signifies astrocyte activation . (E) represents the control brain image, while (F), (G), and (H) show neurons and astrocytes in a mild TBI at the 7, 14, and 28 day marks, respectively. (I), (J) and (K) show neurons and astrocytes in a moderate TBI, while (L), (M), (N) represent severe TBI, all at the same 7, 14, and 28 day time marks.

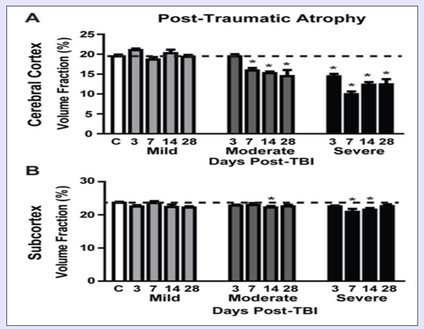

Figure 3: **Quantification of Cerebral and Sub Cortex Atrophy from Image J**
(A) shows the atrophy of the cerebral cortex at the 3, 7, 14, and 28 day marks in the mild, moderate, and severe injury models, while (B) shows the atrophy of the sub cortex.

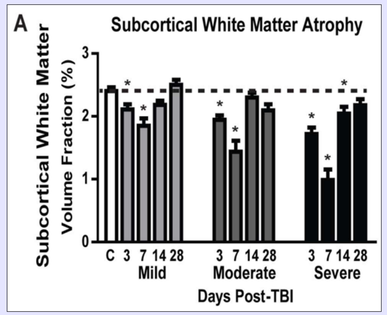

Figure 4: **Quantification of White Matter Atrophy from Image J**
Atrophy of the white matter at the 3, 7, 14, and 28 day marks in the mild, moderate, and severe injury models.

Results & Conclusion

Fluorescence Imaging:
Imaging analysis determined that GFAP and CD68 expression occurred in the cerebral cortex and sub cortex at all degrees of injury. The presence of GFAP and CD68 indicates the activation of glial cells, astrocytes and microglia in these areas, and thus, glial scarring occurred in even the mildest TBIs.

Quantification of Atrophy:
Further analysis of the imaging data in the cerebral cortex and sub cortex indicates that no significant atrophy occurred with mild TBI (similar to a mild concussion), whereas the moderate and severe injuries show significant atrophy in both the cortices. Atrophy in the white matter, however, was significant at all degrees of injury severity at the 3 and 7 day marks. This significant amount of atrophy could be caused by brain sheering of the white matter due to the movement of the brain inside the skull during the administration of the injury., which helps strengthen this model for realistic data on TBIs.

Confocal Microscope Imaging:
The data collected from the confocal microscope imaging exposed a correlation between neuronal death and glial activation. With cell counting, it was determined that areas with higher concentrations of activated astrocytes had a lower number of neurons, further implying that the activation of astrocytes in even the mildest injury may correlate with neuronal death.

Conclusion:
From this research, it was determined that glial cells are activated in all degrees of TBI. The discovery that glial activation is taking place in mild TBI is especially noteworthy, as it implies that significant brain damage can occur in seemingly small head traumas. By knowing the level of injury at which significant damage occurs, more importance can be placed on TBI safety and prevention. Finally, future studies of this nature could have implications in sports and war related brain injuries.

References

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Zeguang, R., Iliff, J. and Nedergaard, M. 'Hit & Run' Model of Closed-skull Traumatic Brain Injury (TBI) Reveals Complex Patterns of Post-traumatic AQP4 Dysregulation. *Journal of Cerebral Blood Flow and Metabolism* 2013; 1-18.

Acknowledgements

High Point University, Department of Chemistry
Dr. Elizabeth McCorquodale , Ms. Megan Perry
University of Rochester, Center for Translational Neuromedicine
Maiken Nedergaard, Jeffrey J Iliff, Zeguang Ren, Lijun Yang, Xiaolin Chen, Michael J Chen, Baozhi Wang, Xuefang Shi, Jainkai Yang