

# Early Metabolic Changes Following Focal Traumatic Brain Injury in Rats Measured Using $^1\text{H}$ MRS

S. Xu<sup>1,2</sup>, S. Roys<sup>1,2</sup>, J. Racz<sup>3</sup>, D. Shi<sup>1,2</sup>, J. Zhuo<sup>1,2</sup>, R. Gullapalli<sup>1,2</sup>, and G. Fiskum<sup>1,3</sup>

<sup>1</sup>Core for Translational Research in Imaging @ Maryland (C-TRIM), University of Maryland School of Medicine, Baltimore, MD, United States, <sup>2</sup>Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, Baltimore, MD, United States, <sup>3</sup>Anesthesiology and the Center for Shock Trauma and Anesthesiology Research, University of Maryland School of Medicine, Baltimore, MD, United States

## Introduction

Traumatic brain injury (TBI) is characterized by acute physiology changes that may play a significant role in the final outcome resulting from such an injury. Experimental models of TBI provide a useful tool for understanding the very early cerebral metabolic changes induced by the damage. Previous *in vivo*  $^1\text{H}$  MRS studies indicated a time evolution of TBI [2-3]. Schuhmann *et al* showed that total creatine (tCr), *N*-acetylaspartate (NAA), glutamate (Glu), and choline (Cho) concentrations significantly decreased during the first 24 hour, and then started to increase at 7 days. At the same time, lactate (Lac) increased and reached its peak at 7 days after TBI. Because the early neuro-metabolic changes may offer valuable information for the clinical neuroprotective treatment, in the present study, we investigate the post-traumatic neuro-metabolic changes at 3-hours and 5-hours after TBI following a focal controlled cortical impact injury in rat, using *in vivo*  $^1\text{H}$  MRS at 7 Tesla.

## Materials and Methods

### TBI Model

Six adult male Sprague-Dawley rats (300-350 gms) were subjected to left parietal controlled cortical impact injury [1]. After being anesthetized initially with 4% isoflurane, the rats were maintained at 2% isoflurane, and the left parietal bone was exposed via a midline incision in a stereotactic frame. A high-speed dental drill was used to perform a left-sided 5 mm craniotomy that was centered 3.5 mm posterior and 4 mm lateral to bregma. A 5 mm round impactor tip was accelerated to 5 m/sec with a vertical deformation depth of either 1.0 or 1.5 mm and impact duration of 50 ms. The bone flap was immediately replaced with dental acrylic and the scalp incision was closed with 3.0 silk. The experimental protocol was approved by the Committee for the Welfare of Laboratory Animals of the University of Maryland.

### In Vivo $^1\text{H}$ MRS

All experiments were performed on a Bruker Biospec 7.0 Tesla 30 cm horizontal bore scanner using Paravision 5.0 software. A Bruker  $^1\text{H}$  surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. Proton density-weighted MR images were taken using a 2D rapid acquisition with relaxation enhancement (RARE) sequence (TR/TE=5500/9.5 ms) for anatomic reference. A point-resolved spectroscopy (PRESS) pulse sequence (TR/TE=2500/20 ms) was used for data acquisition from a  $3 \times 3 \times 3 \text{ mm}^3$  voxel. The voxel covered immediate pericontusional zone, all layers of the hippocampus, and superior thalamic structures. Data were acquired before injury (baseline), at 3-hours, and at 5-hours after injury in both pericontusional voxel (Fig 1A) and the corresponding contralesional side (Fig 1B). For each spectrum, 300 acquisitions were averaged for a total of 13 min. At all times during the experiment, the animal was under 1-2% isoflurane anesthesia and 1 L/min oxygen administration. Respiratory monitoring was performed and the animal was maintained at 36-37°C during the entire experiment.

Proton MRS data was fitted using the LC Model package, and only metabolites with standard deviations (SD) % < 20 were included for further analysis. The *in vivo* mean metabolite concentrations relative to tCr at each time point were subjected to paired one-tail Student t-test in comparison with the control time point.

## Results

Fig 1 demonstrates the bilaterally anatomic images with the voxel located in the axial view (A and B) and the corresponding bilateral spectra (C and D) at 3-hours after TBI from a rat brain. The *in vivo*  $^1\text{H}$  spectra demonstrate good spectral resolution and sensitivity both at the pericontusional side and the contralesional side. Among the metabolic ratios of, NAA/tCr, Glu/tCr and Cho/tCr demonstrated significant changes over the five hours following injury as shown in Fig 2. No statistically significant differences were found in glutamine, myo-inositol, and taurine concentrations among the three time points in either the pericontusional voxel itself or in comparison to the contralesional side.

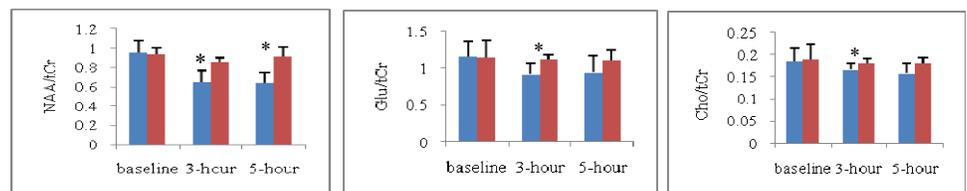


Fig 2 Neuro-metabolic (NAA, Glu and Cho) levels in the TBI rat before injury (baseline), at 3-hours, and at 5-hours after injury. Blue bars indicate pericontusional side; red bars indicate the contralesional voxel; “\*” indicates  $p < 0.05$ .

Significant reduction of 32 % and 33 % NAA was observed in the pericontusional voxel at 3-hours and 5-hours after TBI respectively compared to the baseline. Although the contralesional voxel also exhibited significant reduction in NAA this reduction was much lower compared to the pericontusional side. No significant differences in NAA were found in the pericontusional side between the 3 and 5 hours. In addition to NAA, our results showed that Glu significantly decreased at 3-hours after TBI in the pericontusional voxel, compared to the baseline ( $0.922 \pm 0.137$  vs.  $1.155 \pm 0.202$ ,  $p < 0.03$ ) and the contralesional side ( $0.922 \pm 0.137$  vs.  $1.12 \pm 0.08$ ,  $p < 0.04$ ). As with NAA, we did not observe a significant difference between 3-hours and 5-hours in Glu level in the pericontusional side. Cho in the pericontusional voxel was significantly lower than the contralesional side ( $0.166 \pm 0.014$  vs.  $0.179 \pm 0.013$ ,  $p < 0.05$ ) at 3-hours after TBI. Although, the signal intensities of Lac were undetectable during the baseline, varying levels (0.115 - 2.098) of increased Lac signal intensity was observed in the pericontusional voxel at 3-hours and 5-hours after the injury, but not in the corresponding contralesional voxel.

## Discussion

This study shows that there exists a temporal window of brain vulnerability after TBI in rat, which is in line with previously studies [2]. Furthermore, our investigation demonstrates that the neuro-metabolic changes following TBI associated with NAA, Glu and Cho may have their most significant changes as early as three hours after the injury. Since the pericontusional voxel chosen in this study is of special clinical interest for neuroprotective treatment strategies, our finding may indicate a temporal window of about 3 hours for planning interventions that target cerebral energy metabolism.

## Acknowledgement

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## References

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