Non-invasive magnetic resonance spectroscopy biomarkers of oxidative stress following traumatic brain injury

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Introduction

Traumatic brain injury (TBI) is a major public health concern. At least 1.5 million head injuries occur each year in the United States alone, with annual costs approaching $US60 billion. Although many of these individuals make good recovery, a substantial fraction retain persistent cognitive deficits that impact their lives and those of their families. Despite this enormous financial and social cost, no effective treatment is available for human survivors of TBI. One reason for this shortfall is thought to be the absence of reliable and robust biomarkers of injury that might facilitate translation of compounds that are promising in animal TBI models to human trials.

Magnetic resonance spectroscopy (MRS) provides a quantitative measure of numerous neurochemicals that are associated with specific cellular and molecular mechanisms and which are altered following traumatic brain injury in humans and animals. Indeed, studies from our group have shown that post-TBI levels of N-acetylaspartate, a neuronal marker, predict cognitive recovery in humans [1]. Using high-field MRS, two anti-oxidant species, glutathione (GSH) and vitamin C (Asc; ascorbate), can be quantified in the brain. Since oxidative stress is increasingly implicated as a pathological mechanism following TBI, anti-oxidants are currently under intense investigation as a therapy for brain injury. The goal of the current study was to characterize the temporal evolution of GSH and Asc in a well-characterized rat model of traumatic brain injury. We probed two brain locations: one proximal to the injury site to investigate focal effects and a second, more distal, to investigate possible diffuse brain injury.

Methods

Adult male Fischer 344 rats (n=9) were subjected to unilateral controlled cortical impact (CCI) of the sensorimotor cortex. Injury parameters were: impact tip size = 5mm; velocity = 3.5m/s; depth = 2.0mm; contact time = 300ms. A Varian 9.4T spectrometer was used to collect MR spectra from two voxels using a water-suppressed STEAM sequence (TE=2ms, TR=4000ms; [2]). The first voxel (3 x 2.5 x 3mm^3) was selected in hippocampus to ensure that it would be entirely filled by tissue even after the contusion cyst had developed i.e., consistent with findings in humans where the voxel was located in radiologically normal-appearing brain tissue adjacent to the injury. The second voxel (2.7 x 1.3 x 2.7 mm^3) was chosen in cortex immediately adjacent (ventral) to the impact site to examine more severely injured tissue. First and second order shims were adjusted using FASTMAP [3]. Spectra were analyzed using LCModel [4]. Baseline NAA, GSH, and Asc levels were quantified prior to injury and on days 0 (1-2 hrs post-injury), 1, 3, 7, and 14 post-injury. Results were expressed as a percent of pre-injury baseline.

Results

High quality spectra were obtained consistently from the hippocampal voxel, which was located more remotely from the impact site. Adequate quality spectra (based on signal-to-noise and linewidth criteria) were obtained from the cortical location on days 1 and 3 post-injury. Overall the magnitude of spectral changes was considerably greater in the cortical location.

N-acetylaspartate (NAA): In both voxels, NAA fell rapidly following TBI. In the hippocampal location, NAA levels fell to about 90% of pre-injury levels within 2 hours of TBI whereas in the cortical location NAA fell to 60%. NAA levels continued to fall until day 3. By day 14, hippocampal NAA had recovered to >90% of pre-injury baseline.

Glutathione (GSH): GSH fell rapidly following TBI. In the hippocampal location, GSH levels fell to about 90% of pre-injury levels within 2 hours of TBI and continued to fall until day 3. On days 7 and 14 hippocampal GSH had recovered to ~90% of pre-injury baseline. Cortical GSH spectra did not meet quality criteria at any time point preventing quantification close to the injury site.

Vitamin C (Asc): In both voxels, Asc fell rapidly following TBI. In the hippocampal location, Asc levels fell to about 90% of pre-injury levels within 2 hours of TBI. By day 3, hippocampal Asc levels had returned to baseline and remained in the normal range for the subsequent time points. In contrast, cortical Asc had fallen to about 45% on day 3.

Discussion

The presence of a neuronal injury was confirmed by the NAA results that showed a rapid fall immediately after injury. Significantly lower than normal levels persisted until day 3 followed by partial recovery. This time-course is similar to previous TBI studies in brain slices [5].

We discovered significant alterations to the anti-oxidant system with both Asc and GSH falling rapidly after injury. However, although GSH reductions persisted for several days in the hippocampus, Asc recovery appeared to be initiated within 1 day of injury. Our results are consistent with previous studies of anti-oxidants using invasive methods following TBI.

The reasons for unsuccessful cortical acquisitions remain unclear later in the recovery time course, although it is likely that magnetic field inhomogeneity resulting from the growing contusion cyst and evolving blood products are the source. Nonetheless, the considerably greater magnitude of neurochemical changes in the cortical location will provide increased sensitivity to detect possible beneficial effects of anti-oxidant therapy. Since both GSH and Asc can be quantified using MRS in humans, this approach might provide a novel approach to selecting participants for trials of new medications in survivors of TBI.

References: 1. Friedman SD, et al., Neurology, 1999, **52**: 1384-91. 2. Tkac I, et al., Magn Reson Med, 1999, **41**: 649-56. 3. Gruetter R, Magn Reson Med, 1993, **29**: 804-11. 4. Provencher SW, Magn Reson Med, 1993, **30**: 672-9. 5. Gasparovic C, et al., J Neurotrauma, 2001, **18:** 241-6.