

MRI assessment of iron-mediated pathology following juvenile traumatic brain injury

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Introduction

Pediatric traumatic brain injury (TBI) is the leading cause of morbidity and mortality in children and adolescents. Given development of iron acquisition and mobilization systems in the immature brain, there is an age-dependent susceptibility to iron dysregulation following juvenile TBI (jTBI). The present study characterized iron mediated neuropathology in a rat model of graded jTBI using multi-modal MRI that correlated with histological measures.

Methods and Materials

Graded jTBI: Cortical controlled impact (CCI) was conducted in male Sprague Dawley juvenile rat pups (PND17) to generate jTBI. Graded severities were obtained using variable impact depths, 1 mm for mild, 1.5 mm for moderate and 2 mm for severe injury. Sham animals underwent the same surgical procedures without CCI. **Neuroimaging and Analysis:** At 6h and 1, 3, 7d after surgery, susceptibility weighted imaging (SWI) was acquired on a 4.7T MRI (Bruker Biospin) and diffusion tensor imaging (DTI) was obtained ex vivo on 11.7T MRI. SWI data was post-processed into high pass filter phase and minimum intensity projection (MIP) images using in-house software (Spin). 3D imaging software (Amira, TGS Template Graphics Software, Inc.) extracted lesion volume data from the MIP images. DTI data were processed using an in-house Matlab routine and relative anisotropy (RA) was quantified. The regions of interest (ROIs) included ipsilateral cortex and corpus callosum (CC). **Tissue histology:** Nuclear red staining was performed to confirm the severity of injury and white matter injury was identified by silver staining.

Tissue iron measures: Prussian blue iron staining was used to localize tissue iron deposition. Non-heme iron concentration in dissected brain regions were measured by graphite furnace atomic absorption spectrometry (GFAAS; SpectrAA 220Z).

Results

3D reconstruction of SWI images revealed an increasing jTBI lesion volume along with increased CCI severity, which was confirmed by histology (Fig. 1). After injury, there was a persistent decrease in the SWI phase values within the CC at all times except at 1d. White matter injuries were evident within the CC with a 50% reduction in RA at 6h after jTBI until 7d, consistent with observed axonal damage (Fig. 2). Prussian blue staining showed iron accumulation in the ipsilateral cortex and CC at 3d and 7d following jTBI. Non-heme iron concentrations increased within the ipsilateral frontal cortex, subcortical gray and white matter at 3d after severe jTBI (Fig. 3).

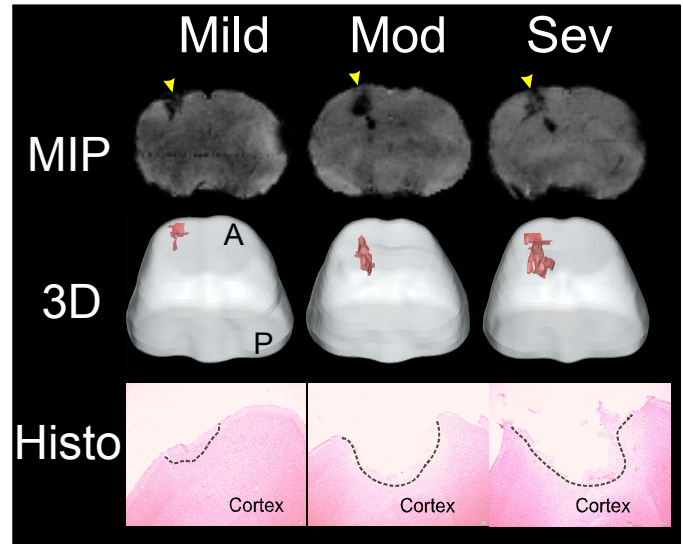


Fig. 1 SWI assessments of traumatic injury severity at 6h after graded jTBI, correlated to histology. Panel 1: The lesion size (arrow head on MIP images) increases with increased CCI severity (depth of impact), consistent with iron accumulation; Panel 2: 3D reconstruction of the lesion volume increases as CCI severity increases from mild to moderate (Mod) to Severe (Sev); Panel 3: Histological staining (nuclear red) shows graded jTBI severity. A=Anterior; P=Posterior; Histo=Histology.

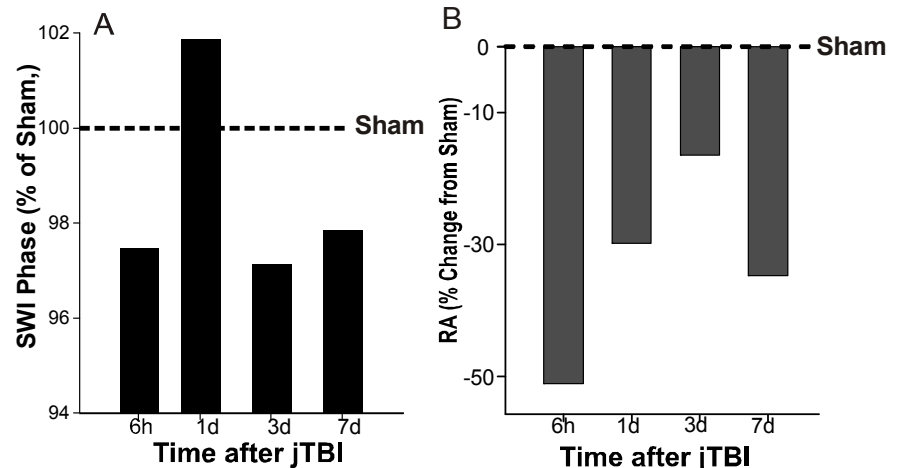


Fig. 2 Temporal MRI within the corpus callosum distant (2mm) from impact site following severe jTBI. A) There was a persistent decrease in phase at all times except for 1d post-jTBI that correlated with iron accumulation; B) Consistent decreases in RA revealed white matter injury over 7d.

Conclusions

SWI is sensitive to monitor pathological iron accumulation *in vivo* following jTBI and correlates with increased tissue iron deposition, especially, non-heme iron. The iron-mediated neuropathology is dominant in the corpus callosum at this age.

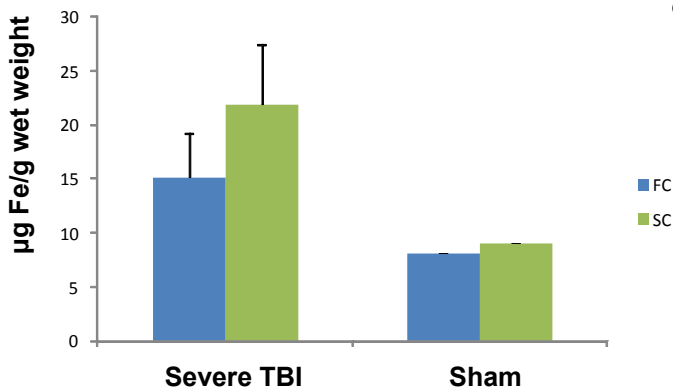


Fig. 3 Non-heme iron concentrations within ipsilateral frontal cortex (FC), subcortical gray and white matter (SC) at 3d after severe jTBI. Compared to sham animals, non-heme iron concentrations were consistently higher in severely injured brain tissues; FC $p=0.217$ and SC $p=0.145$ vs Sham.