Multi-Modal Neuroimaging and Spectroscopy of Mild and Severe Traumatic Brain Injuries in Rodent Model

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TARGET AUDIENCE: Scientists and clinicians interested in imaging biomarkers for traumatic brain injuries.

INTRODUCTION: Traumatic brain injury (TBI) is a serious and global public health issue. Fluid percussion injury (FPI) in rats is a commonly used animal model simulating traumatic brain injuries. *In-vivo* ^{1}H magnetic resonance spectroscopy (MRS) provides information on cerebral metabolism including N-Acetyl Aspartate (NAA), which is a neuronal marker and total Choline (tCho = Glycerophosphocholine (GPC) + phosphocholine (PCh)) content which is a marker for cell membrane turnover and inflammation. Diffusion tensor imaging (DTI) provides information on micro-structural changes and axonal damage due to injuries related to swelling, inflammation, edema and fiber damage [1, 2]. Susceptibility weighted imaging (SWI)[3] allows improved detection of paramagnetic hemorrhagic blood components based on their magnetic susceptibility and provides information on microvasculature. In this study, we investigated the metabolic changes by MRS (*in-vivo* and *ex-vivo*) and structural changes using DTI in hippocampus and changes in microvasculature by SWI in FPI based mild and severe TBI.

METHODS: Adult male Sprague Dawley rats (280 – 300g) were subjected to focal brain injury using the lateral fluid percussion device. Animals were subjected to either 1) Mild (~22 psi), 2) Severe (~60 psi) injury at 2mm lateral and 3.8mm posterior to bregma. The study set had, 8 mild and 6 severe rats. Single voxel spectroscopy (SVS) based PRESS data (water suppressed and unsuppressed) were acquired before and after the injury with a voxel size of 3.5 × 2 × 3.5 mm^3 in the hippocampus with the TR/TE = 4000/13 ms. DTI was performed using SE-EPI based acquisition with TR/TE/slice thickness/#slice/FOV/matrix size/#direction = 000 ms/50ms/1.2mm/28/36×28mm^2/128×100/20. Four averages were acquired with b-factors of 0 s/mm^2 and 1000 s/mm^2. SWI was performed using TR/flip angle/slice thickness/#slice/FOV/matrix size/# average = 45 ms /20°/0.8 mm/64/36 mm × 27 mm/512×384/2. A total of 5 echoes were acquired with a central-echo time of 14.95 ms and an echo-spacing of 4.008 ms. MRI/MRS data were acquired using 7T Bruker ClinScan equipped with 4-channel RAPID phased array coil at baseline (BL, before the injury) and at 3hours, day 1, 3, 7, 14 after the injury. ^{1}H high-resolution magic angle spectra (HRMAS) of brain tissue samples were acquired using 9.4T Bruker Avance III spectrometer. 1D spectra were acquired using Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence, data points-32K, spectral width-7 kHz, relaxation delay-7 sec, transients-128 (with water pre-saturation). In vivo MRS and ex vivo HRMAS data were analyzed using LC model [4] and TOPSPIN 3.0 respectively. A Java based ImageJ plugins were developed for processing and analysis of DTI and SWI data [5, 6].

RESULTS AND DISCUSSIONS: Fig. 1(A) and (B) show the NAA/tCr and tCho/tCr at different time points including baseline and after mild and severe injuries. Significant reduction of NAA due to neuronal loss is observed in the case of severe injuries whereas mild injuries showed moderate reduction [7] on day 1. There is also a modulation of the NAA concentrations in severely injured animals due to secondary injuries. Massive efflux of excitatory neuro transmitter glutamate from the presynaptic neuron may trigger a high influx of calcium into the postsynaptic neuron. This high influx of calcium may cause the phospholipid membrane breakdown [8]. After TBI, there is a breakdown of blood brain barrier as early as 1hr after the trauma, which closes again after 6hrs from the injury [9]. tCho is a cell proliferation and degradation marker, is reduced significantly within 3 hours after the severe injury (Fig. 1B) which is also confirmed by *ex-vivo* HRMAS (Fig. 1C). Fig 1D shows the hippocampus mean diffusivity (MD) with a significant increase during day 1 for both mild and severe injuries, which decreased during subsequent days. The increase in the MD is due to the increase in the volume of extracellular fluid and/or cellular membrane disruption [10]. Fig. 2 shows the color-coded eigenvector overlaid MD maps. Fig. 3 shows the combined filter phase and minimum intensity projection (mIP) over 7 slices of SWI for mild (top row) and severe (bottom) injuries. There is a decrease in the signal in vessels after mild and severe injuries due to increase in deoxyhomoglobin[11]. The derived R2* map computed from echoes is shown in Fig 4.

CONCLUSIONS: Neuronal loss and membrane disruption is observed in mild and severe injuries by in-vivo and ex-vivo MRS. DTI provided micro-structural changes in hippocampus through mean diffusivity. Multi-echo SWI provided the phase and R2* which can be utilized to investigate calcification, haemorrhage and measure of iron deposition in the brain [6]. The decrease in the signal from the micro-vessels after injury is due to the increase in deoxyhemoglobin. The relative changes in cerebral blood flow can be estimated using filter phase images by utilizing the changes in oxygen saturation [5]. The combined imaging and spectroscopic assessment provides valuable markers for investigating structural and metabolism in mild and severe injuries.

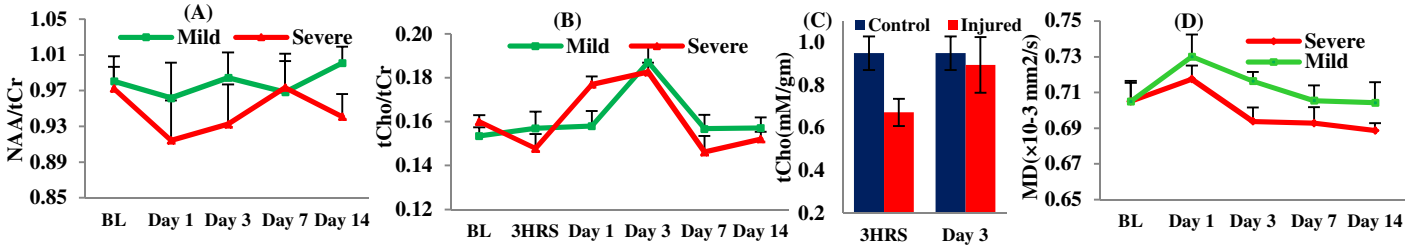

Fig. 1. **(A)** Ratio of NAA to tCr, **(B)** tCho to tCr, **(C)** tCho concentration by HRMAS and **(D)** hippocampus MD at different time points.

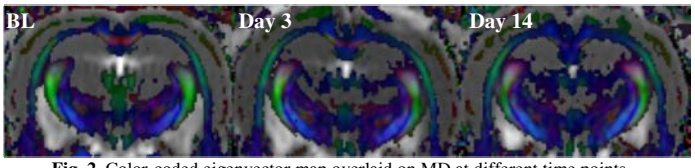

Fig. 2. Color-coded eigenvector map overlaid on MD at different time points.

References: **1.** Basser PJ, *et al*. JMR 111, 209–219, 1996. **2.** Alexander AL, *et al*. Neurotherapeutics 4(3):316-29, 2007. **3.** Haacke EM, *et al*. MRM 52:612-618, 2004. **4.** Provencher *et al*. NMR Biomed 14:260–264, 2001. **5.** Rathore RK, *et al*. MRI 29(8):1088-100, 2011. **6.** Verma SK, *et al*. ISMRM 2013, #1072. **7.** Choi CB, *et al*. Eur J Radiol 55(1):96-101, 2005. **8.** Klein J, *et al*. J Neural Transm, 107:1027-1063, 2000. **9.** Härtl R *et al*. Acta Neurochir Suppl 70:240-2, 1997. **10.** Mac Donald CL, *et al*. Exp Neurol 205, 116–131, 2007. **11.** Shen Y, *et al*. MRI 25(2):219-27, 2007.

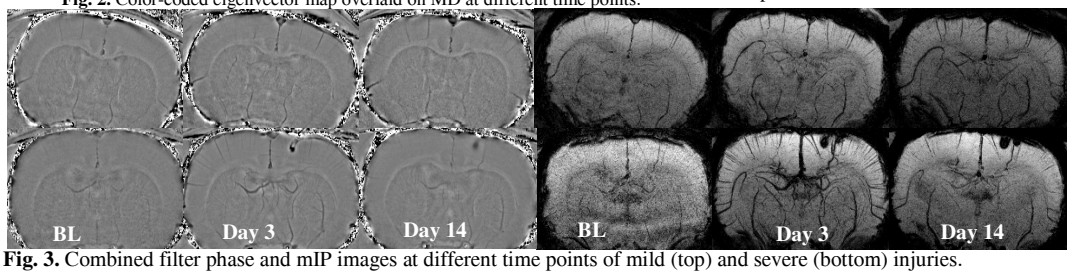

Fig. 3. Combined filter phase and mIP images at different time points of mild (top) and severe (bottom) injuries.

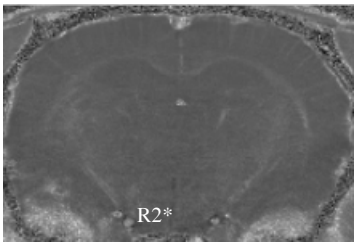

Fig. 4. R_2* map derived from 5 echoes.