Understanding the interplay different MRI methods have as white matter changes longitudinally in the cuprizone mouse model

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INTRODUCTION: Mouse brain white matter (WM) damage following administration of cuprizone (CPZ) was studied *in vivo* weekly using DTI[1], qMTI[2], T_1/T_2[3] relaxometry, T_2-weighted (T2w) MRI, and electron microscopy (EM). A previous study examined correlations between MR metrics and EM measures after 6 weeks of feeding[4]. The addition of weekly *ex vivo* tissue analysis allows for a more complete understanding of the correlations between MR metrics and EM measures of tissue pathology. **METHODS:** ***Mouse Model*** C57BL/6 mice (n=18 control (CTL) and 18 experimental) were fed 0.3% CPZ (w/w) starting at 8 weeks of age. After each week of feeding, a subset of mice was perfused with phosphate buffered saline (PBS) followed by 0.5% glutaraldehyde and 2% paraformaldehyde followed by PBS as done previously[4]. All tissue external to the skull was removed and the mouse head was stored in PBS prior to overnight imaging. All experiments were approved by the university's Animal Care Committee. ***MRI*** MRI was performed on a 7T Bruker Avance III MRI system. Mice were anesthetized using 1.5% isoflurane in O_2/N_2O. In order to reduce volume averaging effects, coronal slices were selected perpendicular to the rostral region of the corpus callosum (CC)[4]. Initially, mice underwent *in vivo* T2w and MTI on the day the treatment began (week 0) and one week later (week 1). Starting on week 1, 6 animals (3 CTL, 3 CPZ) were sacrificed each week for *ex vivo* analysis. After sacrifice, additional high-resolution T2w, DTI, qMTI, and T_1/T_2 relaxometry datasets were acquired. *In vivo* T2w and MT images were aligned using manual and mutual information image registration[5]. ROIs representing both the CC and the external capsule (EC) were selected in the *in vivo* MT contrast images and *ex vivo* directionally encoded color maps (DEC) maps and applied to analysis of all images. All images were acquired on the same coronal slice with 98x98x750 μm^3 resolution. FOV/matrix size was $(2.5\ cm)^2$/256x256 *in vivo* and $(1.25\ cm)^2$/128x128 *ex vivo*. ***In vivo T2w*** RARE, NA=12, TE_{eff}/TR=80/1640 ms, RARE factor 8, 10 min. ***In vivo MTI*** FLASH, NA=48, TE/TR=6/70 ms, 10° flip angle, one with an MT saturation pulse (Gaussian, 10.25 ms, 10 μT, 6 kHz off-resonance) and one without an MT saturation pulse, 2x14 min to calculate MTR maps. ***Ex vivo T_1/T_2 Relaxometry*** Fit to a series of RARE images, TE_{eff}=11, 33, 55, 77, 99 ms; TR=5, 3, 1.5, 0.8, 0.4, 0.353 s; RARE factor 2; NA=8; 71 min. ***Ex vivo qMTI*** 1 proton density image + 18 MTIs (5, 10, and 20μT and frequency offsets at each power of 1, 2, 4, 6, 10, and 30 kHz) NA=64 19x9.6 min; ***Ex vivo DTI*** PGSE, tetraorthogonal gradient-encoding scheme, b-value=1000 s/mm^2 (δ=6 ms, Δ=14 ms), NA=6, TE/TR=26/5000 ms, 5 hr. ***Ex vivo T2*** RARE, NA=36, TE_{eff}/TR=80/1640 ms, RARE factor 8, 31 min. Following MRI, the brains were returned to 2% glutaraldehyde, then the CC was dissected and embedded in epoxy resin for EM. Correlation between both longitudinal and quantitative datasets was measured in the CC and EC. **RESULTS Figure 1** Representative normalized *in vivo* (a,b) and *ex vivo* (c) MR data from weeks 0-6. Week 0 *ex* vivo image is from a CTL. Signal inversion is apparent in the T_2w image (a) and decreased MTR (b) is observed in the WM regions of the CPZ mouse (arrows). Directionality changes in the EC are apparent in DEC (c) map from weeks 1 to 6. **Figure 2** Weekly changes (mean ± SEM) in the CC (black) and EC (blue) in the CPZ (dotted) and CTL (solid) mice. Data from *in vivo* images (T2w & MTR) are shown for weeks 0-6. Data for *ex vivo* images ($\lambda_\perp$) are shown for weeks 1-6. **Figure 3** EM of CTL and CPZ mice CC at week 3. Myelinated axons are apparent in both. CPZ is associated with oligodendroglial swelling and apoptosis. The significant change between CTL and CPZ mice in the CC peaks in T_2 at week 3 whereas it peaks at week 4 in MTR. The first large change in T2w occurs between weeks 2 and 3 in the EC and between weeks 3 and 4 in the MTR. $\lambda_\perp$ appears to be different between CTL and CPZ mice even in week 1. The weekly changes in $\lambda_\perp$ follow a different time course than MTR and T_2 in the CPZ mouse. **DISCUSSION & CONCLUSION** The different time courses of the MR metrics suggest that T_2 and MTR are sensitive to different pathological features in WM. ANOVA will be used to determine when significant changes occur in MRI metrics. EM analysis of the tissue is in progress for correlations with WM pathology. Visually it can be seen in the above EM images at week 3 that the CTL and CPZ CC show a similar amount of myelinated axons. Our results are consistent with EM from other studies[4] suggesting MTR likely reflects demyelination. The addition of the weekly *ex vivo* tissue analysis allows for a more complete understanding of the correlations between MR metrics and EM measures of tissue pathology. **REFERENCES**: 1 Song, NeuroImage 26:2005. 2 Tozer, MRM 50:2003. 3. Laule, NeuroImage 40:2008. 4. Thiessen, NMRBiomed 26:2013. 5. Pluim, IEEE Trans Med Imag 22:2003. **FUNDING**: NSERC, MHRC, CFI, & MRIF