Correlating longitudinal and quantitative MRI metrics elucidates white matter changes in the cuprizone mouse model of demyelination

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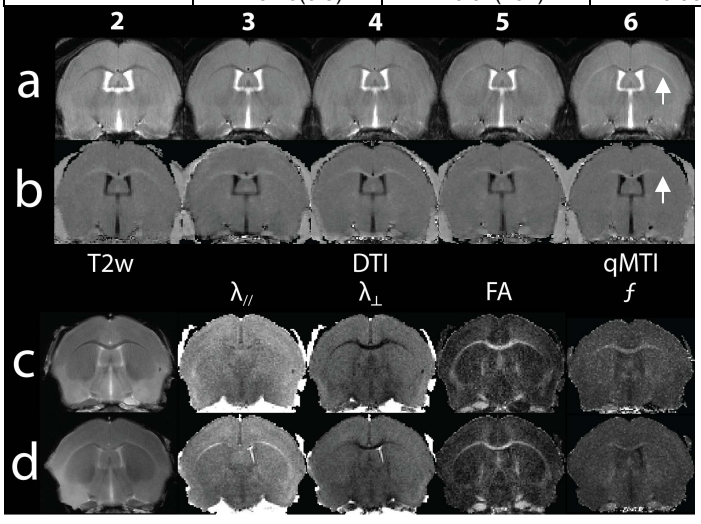
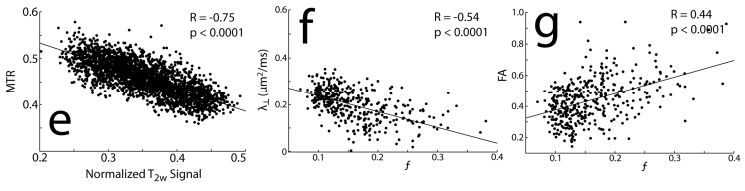
INTRODUCTION: MRI methods such as diffusion tensor imaging (DTI)[1], quantitative magnetization transfer imaging (qMTI)[2], and multicomponent T_2 relaxometry[3] may help quantify changes related to demyelination. To understand the interplay different MRI methods have as white matter changes longitudinally in the cuprizone mouse model, *in vivo* T_2-weighted (T2w) and magnetization transfer images (MTI) were acquired weekly in control (CTL) and cuprizone-fed mice (CPZ). As well, DTI, qMTI, T_1/T_2 relaxometry, T2w imaging, and histopathology were used to analyze *ex vivo* tissue after 6 weeks of cuprizone delivery. Correlation between both longitudinal and quantitative datasets was measured with a focus on the corpus callosum (CC).

METHODS: ***Mouse Model*** C57BL/6 mice were fed 0.4% cuprizone (w/w) starting at 8 weeks of age. After 6 weeks of feeding, mice were perfused with PBS/saline solution followed by 4% PFA. Heads were fixed for 24 hours in 4% PFA. 48 hours prior to *ex vivo* imaging, brains were transferred to a PBS solution to leach out the remaining PFA. All experiments were approved by the university's animal care committee. ***MRI*** Experiments were performed on a 7T Bruker Avance III NMR system. Mice were anesthetized using 1.5% isoflurane in O_2/N_2O. Respiration and external body temperature were monitored during imaging. In order to reduce volume averaging effects, coronal slices were selected in each mouse perpendicular to the rostral region of the CC. 4 CTL and 4 CPZ mice underwent *in vivo* T2w imaging and MTI at 2, 3, 4, 5 and 6 weeks after start of treatment. 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[4]. Regions of interest representing both medial and lateral regions of the CC as well as the cerebral cortex were selected in the T2w images and applied to analysis of all MR methods. All images were acquired on the same 3 coronal slices with 1.25 mm inter-slice spacing and 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, 12 averages, effective TE/TR = 80/1640 ms, RARE factor 8, 10 minutes. ***In vivo MTI*** FLASH, 48 averages, TE/TR = 6/70 ms, 10° flip angle. In order to calculate the magnetization transfer ratio (MTR), images were acquired with an MT saturation pulse (Gaussian, 10.25 ms, 10 μT, 6000 Hz off-resonance) and without an MT saturation pulse, 2x14 minutes. ***Ex vivo T_1/T_2 Relaxometry*** Fit to a series of RARE images, effective TE = 11, 33, 55, 77, 99 ms; TR = 5000, 3000, 1500, 800, 400, 353 ms; RARE factor 2; 8 averages; 71 minutes. ***Ex vivo qMTI*** 1 proton density image + 24 MT images acquired with irradiation powers of 5, 10, and 20μT and frequency offsets at each power of 100, 300, 1000, 2000, 4000, 6000, 10000, and 30000 Hz. ***Ex vivo DTI*** PGSE, tetra-orthogonal gradient-encoding scheme (7-directions), b-value = 1000 s/mm^2 (δ = 6 ms, Δ = 14 ms), 1 slice, 6 averages, TE/TR = 26/5000 ms, 2.5 hours. ***Ex vivo T2w*** RARE, 1 slice, 36 averages, effective TE/TR = 80/1640 ms, RARE factor 8, 31 minutes. ***Histopathology*** 30 μm sections were stained with either Luxol fast blue-periodic acid Schiff (LFB-PAS) or immunostained for myelin basic protein (MBP). ***Statistical Analysis:*** Pearson's correlation coefficients were calculated using both mean ROI values and individual voxel values in each registered ROI for CTL, CPZ and combined datasets.

RESULTS & DISCUSSION: LFB-PAS and MBP staining confirmed demyelination in the CC of CPZ mice. *In vivo* T2w images and MTR maps demonstrated significant differences between CTL and CPZ mice as well as significant week-to-week changes in the lateral CC of the CPZ mice (figures a and b). *Ex vivo* T2w, qMTI, and DTI metrics all demonstrated significant differences between the CC of CTL and CPZ mice (figures c and d)[5]. ROI-based analysis (RBA) yielded different correlations than voxel-based analysis (VBA) (see table). Evidently, care should be taken making inferences based on RBA as opposed to VBA, as this assumes that individual voxels can be represented by the average characteristics of the ROI[6]. There were also different correlations for CTL, CPZ, and combined datasets (see table). Correlation between normalized T2w signal and MTR in the medial CC was lower than correlation in the lateral CC (see table, figure e). Weak correlation in the medial CC could be due to intercompartmental water exchange influencing T_2 relaxation[7] while stronger correlation in the lateral CC may suggest common factors that can influence both T2w signal and MTI, such as inflammation or increased extracellular space. VBA correlations between axial diffusivity ($\lambda_\perp$) or fractional anisotropy (FA) and the qMTI macromolecular pool-sizes (*f*) were generally lower in the medial CC compared to the lateral CC with the distinct exception of the CPZ group, where correlation was *higher* in the medial CC (see table, figures f & g). Much like the *in vivo results*, this may suggest factors other than demyelination are affecting the lateral CC results. Since CPZ affects thinner and less mature myelin sheaths[8], lateral regions of the CC may have greater longitudinal changes. In the lateral CC of CPZ mice, significant week-to-week changes and significantly higher axial and radial diffusivity and lower *f* may suggest influential factors beyond just demyelination such as inflammation, increased extracellular space, and/or gliosis[9]. A non-zero FA result at *f*=0 is indicative of structures other than myelin influencing diffusion anisotropy (similar to Mädler *et al.*'s findings when correlating the FA to the myelin water fraction[10]). In conclusion, correlation of both longitudinal and quantitative MRI metrics may help elucidate white matter changes beyond the application of individual MRI methods.

Correlation R(p)	T2w/MTR: CTL	T2w/MTR: CPZ	T2w/MTR: Combined	DTI/qMTI: CTL	DTI/qMTI: CPZ	DTI/qMTI: Combined
Medial CC	VBA -0.37(10^{-41}) RBA 0.09(0.7)	VBA -0.08(0.005) RBA -0.38(0.09)	VBA -0.40(10^{-87}) RBA -0.81(10^{-10})	$\lambda_\perp$/*f* -0.14(0.03) FA/*f* 0.20(0.001)	$\lambda_\perp$/*f* -0.22(0.001) FA/*f* 0.33(10^{-7})	$\lambda_\perp$/*f* -0.28(10^{-10}) FA/*f* 0.33(10^{-13})
Lateral CC	VBA -0.52(10^{-89}) RBA 0.16(0.5)	VBA -0.68(10^{-182}) RBA -0.91(10^{-8})	VBA -0.75(0) RBA -0.90(10^{-15})	$\lambda_\perp$/*f* -0.29(10^{-5}) FA/*f* 0.41(10^{-9})	$\lambda_\perp$/*f* -0.17(0.02) FA/*f* 0.09(0.2)	$\lambda_\perp$/*f* 0.54(10^{-30}) FA/*f* 0.44(10^{-20})





FIGURES: Representative *in vivo* T2w (a) and MTR (b) of CPZ mouse from 2 to 6 weeks of CPZ delivery. Representative *ex vivo* T2w, DTI metrics ($\lambda_{//}$, $\lambda_\perp$, and FA) and the macromolecular pool-size (*f*) for CTL (c) and CPZ (d) mice. VBA correlation in the lateral region of the CC between normalized T2w and MTR (e) and between $\lambda_\perp$/FA and *f* (f and g).

REFERENCES: [1] Song, S-K *et al*. NeuroImage 26:132-140 (2005). [2] Tozer, D *et al*. MRM 50:83-91 (2003). [3] Laule, C *et al*. NeuroImage 40:1575-1580 (2008). [4] Pluim, JPW *et al.* IEEE Trans Med Imag 22:986-1004 (2003). [5] Thiessen, JD *et al.* ISMRM Stockholm:4512 (2010) [6] Steel, DG *et al.* Int Stat Rev 64:39-60 (1996) [7] Dula, A *et al.* MRM 63:902-909 (2010) [8] Yang, H-J *et al*. Brain Research 1270:121-130 (2009). [9] Kolind, SH *et al.* NeuroImage 40:77-85 (2008) [10] Mädler, B *et al.* MRI 26:874-888 (2008).

FUNDING: NSERC Canada, CFI, and MRIF.