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 T2 relaxometry³ 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 T2-weighted (T2w) and magnetization transfer images (MTI) were acquired weekly in control (CTL) and cuprizone-fed mice (CPZ). As well, DTI, qMTI, T1/T2 relaxometry, T2w imaging, and histopathology were used to analyze ex vivo tissue after 6 weeks of cuprizone treatment. 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, 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 O2/N2/O. 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 T1/T2 relaxometry datasets were acquired. In vivo T2w and MTI images were aligned using manual and automated image registration⁴. 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 96x98x750 μm² 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 T1/T2, 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 images + 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 PGE, tetra-orthogonal gradient-encoding scheme (7-directions), b-value = 1000 s/mm² (b = 6 ms, Δ = 14 ms), 1 slice, 6 averages, TE/TR = 26/5600 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).

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). 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. There were also different correlations for CTL, CPZ, and combined dataset (see table). Correlation between normalized T2w signal and MTR in the medial CC was lower than correlation in the lateral CC (see figure e). Weak correlation in the medial CC could be due to intercompartamental water exchange influencing T2 relaxation 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 (λa) 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, 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. A non-zero FA result at f=0 is indicative of structures other than myelin influencing diffusion similar to the FA's finding of relaxing the FA to the media axial diffusion, weak correlation of both longitudinal and quantitative MRI metrics may help elucidate white matter changes beyond the application of individual MRI methods.

<table>
<thead>
<tr>
<th>Correlation R(p)</th>
<th>T2w/MTR: CTL</th>
<th>T2w/MTR: CPZ</th>
<th>T2w/MTR: Combined</th>
<th>DTI/qMTI: CTL</th>
<th>DTI/qMTI: CPZ</th>
<th>DTI/qMTI: Combined</th>
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<tbody>
<tr>
<td>Medial CC</td>
<td>VBA -0.37(0.01)</td>
<td>VBA -0.09(0.05)</td>
<td>RBA -0.38(0.04)</td>
<td>VBA -0.40(0.04)</td>
<td>FA/0.20(0.01)</td>
<td>FA/0.33(0.03)</td>
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<tr>
<td>Lateral CC</td>
<td>VBA -0.52(0.04)</td>
<td>VBA -0.63(0.06)</td>
<td>RBA -0.91(0.09)</td>
<td>VBA -0.75(0.05)</td>
<td>FA/0.41(0.03)</td>
<td>FA/0.98(0.09)</td>
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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 (λa, λr, 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 λa/FA and f (f and g).