Longitudinal study of cuprizone-induced white matter degeneration and recovery using diffusion White Matter Tract Integrity Metrics (WMTI).

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Target audience: Scientists interested in quantifying demyelination and axonal loss in white matter (WM) using diffusion MRI-based tissue models.

Purpose: Monitoring of myelin damage and repair is of great clinical importance for demyelinating diseases, such as multiple sclerosis. Conventional MRI measures, such as T_2, MTR and radial diffusivity (RD), are sensitive, but non-specific to the WM injury, whereas WM tract integrity (WMTI) metrics[1] derived from diffusion kurtosis imaging (DKI) can provide compartment specific estimates of WM properties, such as axonal water fraction (AWF), intra-axonal diffusivity (D_a), extra-axonal axial and radial diffusivities ($D_{e,\parallel}$ and $D_{e,\perp}$). Cuprizone (CPZ) fed mice provide an excellent model of demyelination[2] and the WMTI metrics have been shown to correlate well with histopathology in previous CPZ studies[3]. However, the latter focused on diffusion differences between control and treated at a single timepoint of degeneration (10 weeks). In this study, we quantified *in vivo* changes in both conventional MRI (MTR, T_2, RD) and WMTI parameters during CPZ-induced WM degeneration[2] and subsequent recovery in the mouse corpus callosum.

Methods: With approval from the Institutional Animal Care and Use Committee, we conducted an 18-week longitudinal study on 34 female C57 mice (8 weeks old at baseline) separated into 3 groups: "Control" group received standard chow; "Cpz6" and "Cpz12" groups received CPZ for 6 and 12 weeks, respectively (Fig. 1). **Acquisition:** In vivo MRI was performed using a 7-T Bruker BioSpec magnet. One 0.8-mm thick mid-sagittal slice was obtained with 112-µm in-plane resolution. Diffusion-weighted images were acquired using a 2D GRASE sequence with *b*-values of 1000 and 2000 s/mm^2 and 30 directions each, in addition to 6 *b*=0 images. For T_2 mapping, a multi-echo spin-echo sequence was used (32 echoes, TE step = 7.1 ms). For MTR, two FLASH images were acquired with and without MT saturation pulse (offset = 1.5 kHz; duration = 12 ms; peak power = 4.7 µT). For histopathological evaluation using electron microscopy (EM), subsets of animals were sacrificed and perfusion-fixed at 6 weeks (3 Control, 6 Cpz6), 12 weeks (3 Control, 6 Cpz6, 6 Cpz12) and 18 weeks (3 Control, 6 Cpz12). **Processing:** Multi-echo spin-echo data were fit to a monoexponential decay to generate parametric T_2 maps. The MT-on image was spatially registered to the MT-off image prior to MTR calculation in each voxel. DKI analysis was performed, followed by derivation of the WMTI parametric maps[1]. The splenium was segmented on the color-encoded FA map using a semi-automated in-house software tool. This ROI was also registered to the T_2 and MTR maps. Splenium voxels from all animals within one treatment group at a given timepoint were pooled together for statistical analysis. At each timepoint, a Kruskal-Wallis test evaluated differences between group medians. For each metric, degeneration and recovery in Cpz6 and Cpz12 were quantified by calculating the relative difference between the median values of that group and Control. Pearson correlations at 6 and 12 weeks were also calculated between diffusion metrics, T_2 and MTR.

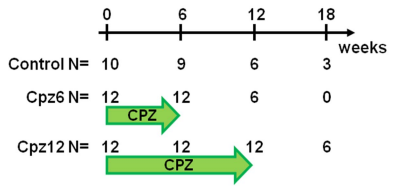

Figure 1. Experimental timeline for different study groups: total number of mice in a group at each timepoint, and duration of diet on CPZ-supplemented chow (0.2%, Sigma Aldrich); otherwise the animals were fed a standard chow diet *ad libitum*.

Results and discussion: **Effect of treatment on metrics**: Figure 2 shows results for RD, $D_{e,\perp}$ and AWF. Given the young age of mice, some developmental changes with time (opposite in trend to the CPZ ones) were seen in the control cohort. At 6 weeks, both Cpz6 and Cpz12 groups (which received identical treatment so far) displayed significantly higher RD, $D_{e,\perp}$, and T_2 (data not shown) than the control group, and significantly lower AWF and MTR (data not shown) (all comparisons p<0.001). At 12 weeks, the Cpz12 group displayed sustained degradation and the Cpz6 group partial recovery in terms of all these metrics. At 18 weeks, the Cpz12 group also displayed partial recovery compared to the 12 week landmark but remained significantly altered relative to Control. Interestingly, in Cpz12, changes in RD and $D_{e,\perp}$ between weeks 6 and 12 were as marked as those between weeks 0 and 6, while changes in AWF, T_2 and MTR were substantially slowed down in the second 6-week period. This suggests that RD and $D_{e,\perp}$ may be more sensitive than AWF, T_2 or MTR to the sustained demyelination (or other type of chronic degeneration) occurring with prolonged exposure to CPZ. However, all changes, except MTR, were partially reversed upon ending the treatment. **Sensitivity**: At 6 weeks, the largest relative difference between Control and Cpz6 or Cpz12 was recorded for MTR (-30%), followed by AWF (-20%). At 12 weeks, the largest relative difference between Control and Cpz12 was seen in RD (+40%) followed by $D_{e,\perp}$ (+30%), due to their sustained increase. **Correlations**: At 6 weeks, T_2 correlated most strongly with MTR, AWF and RD (in this order), and at 12 weeks with RD, AWF, MTR and $D_{e,\perp}$. Regarding MTR, no significant correlations with diffusion metrics were found at 6 weeks; at 12 weeks, MTR correlated with AWF and RD. The strongest correlations overall were found between AWF and T_2 (ρ = -0.55 at 6 weeks and ρ = -0.71 at 12 weeks), consistent with their very similar trends in time.

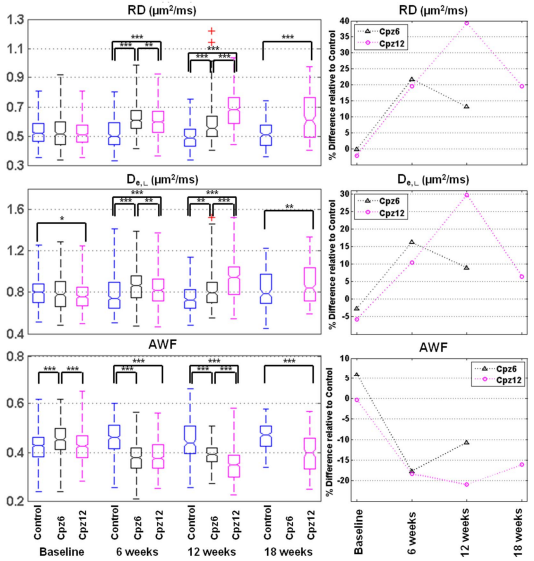

Figure 2. Left: Differences in RD, $D_{e,\perp}$ and AWF between Control (blue), Cpz6 (black) and Cpz12 (magenta) groups. The central mark represents the median, box edges are 25th and 75th percentile; red crosses are outliers. Superimposed are results from the Kruskal-Wallis test of differences between population medians (*: p<0.05; **: p<0.01; ***: p<0.001). Right: Relative difference (in %) in parameter value in Cpz6 and Cpz12 compared to the Control group (based on the medians), for each timepoint.

Conclusion: In this longitudinal study, conventional and WMTI metrics were sensitive to the effect of both CPZ treatment and subsequent recovery. All MRI metrics initially affected by the CPZ treatment (except for MTR) partially recovered at the end of treatment in both Cpz6 and Cpz12 groups. WMTI seems to be able to disentangle between effects of acute and prolonged exposure to CPZ, via the different rates of changes in AWF and $D_{e,\perp}$. Quantification of myelin and axonal volume fraction based on EM image is underway (see Fig.3 as an example) and is expected to provide an independent assessment of microstructural changes at each timepoint for validation, as well as information on which MR metrics correlate best with myelin content and axonal count.

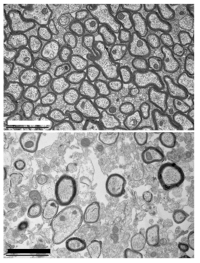

Figure 3. EM images of splenium from a Control mouse (top; myelinated axon density: 1.65 µm^{-2}, myelin volume fraction: 31%) and a Cpz12 mouse at 12 weeks of diet (bottom; myelinated axon density: 0.49 µm^{-2}, myelin volume fraction: 9%). Both severe demyelination and axonal loss are apparent. *Scalebar: 2 µm.*

References: [1] Fieremans et al., Neuroimage 2011. [2] Matsushima and Morell, Brain Pathol. 2001. [3] Falangola et al., NMR in Biomed. 2013. Work supported by NIH R21 NS081230.