Histological correlation of DKI-White Matter Modeling Metrics in the Cuprizone-Induced Corpus Callosum Demyelination

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TARGET AUDIENCE: For those interested in diffusion MRI, white matter diseases and animal models of disease.

PURPOSE: In the cuprizone mouse model, reproducible corpus callosum (CC) demyelination occurs within weeks after mice are fed the copper chelator cuprizone (bis-cyclohexanone oxaldihydrazone), with oligodendrocyte damage followed by demyelination associated with glial response[1]. Recently, a white matter (WM) model has been proposed for relating diffusional kurtosis imaging (DKI) data to specific WM biophysical tissue metrics[2]. Here, we investigate these WM metrics in the CC of the cuprizone mouse model and compare them to values for control mice and to histological markers of myelin integrity.

METHODS: A total of 20 (8–10 weeks old) C57BL/6 mice (n=10 cuprizone-treated: CR, n=10 normal controls: NC) were fed specific diets for 10 weeks. CR mice were fed a diet containing cuprizone (0.2%), (Biscyclohexanone) oxaldihydrazone, Sigma-Aldrich) and NC mice were maintained on a standard diet. All in vivo MRI experiments were performed on a 7T Agilent MR system. A respiration-gated 4-shot SE-EPI sequence was used for DKI acquisition with the following parameters: TR/TE=3000/30ms, δ/Δ=5/17ms, slice thickness=1mm, matrix=128×128, in plane resolution=234×234 μm^2, 4 averages, and 30 gradient directions with five b-values for each direction (0.5, 1, 1.5, 2 and 2.5 ms/μm^2). All metrics were derived from the DKI dataset using Diffusional Kurtosis Estimator[3]. These include 1) the standard DTI-based metrics of fractional anisotropy (FA) and mean (MD), axial ($D_{//}$) and radial ($D_{\perp}$) diffusivity, 2) the kurtosis metrics of mean (MK), axial ($K_{//}$) and radial ($K_{\perp}$) kurtosis, and 3) the WM model metrics of axial and radial extra-axonal diffusivities ($D_{e,||}$ and $D_{e,\perp}$), the intrinsic diffusivity inside the axons (D_a), the axonal water fraction (AWF), and the tortuosity (λ) of the extra-axonal space. To reduce partial volume effects, all parametric maps were masked to eliminate voxels with MD values greater than 1.5 μm^2/ms. After MRI, quantitative analysis of myelin density was performed; all brains were processed and stained with Solochrome (for myelin) at the NeuroScience Associates (Knoxville, TN). Digital pictures of the CC histology slides were obtained and quantitatively assessed based on the mean intensity values from all pixels in three CC regions of interest (ROIs). The ROIs were manually drawn at the level of CC (rostral (aCC), middle (bCC), and caudal (pCC)), for both MRI and histology, using ImageJ (http://rsb.info.nih.gov/ij/). For this analysis, we assumed the mean intensity value to be related to the degree of the histological staining. One-way ANOVA, corrected for multiple comparisons (Sidak) was performed to assess group differences in the ROI measurements between CR and NC mice, with $P \leq 0.05$ considered to be statistically significant.

Table 1:
Mean ± sdev & p-values for the diffusion metrics between NC and CR, for the entire CC.

	FA	MD	$D_{//}$	$D_{\perp}$	MK	$K_{//}$	$K_{\perp}$
Group							
NC	0.35±0.09	0.96±0.05	1.32±0.06	0.78±0.09	0.66±0.06	0.73±0.06	0.71±0.10
CR	0.29±0.06	1.04±0.06	1.35±0.06	0.89±0.09	0.55±0.07	0.65±0.09	0.55±0.13
p-values	0.01	<0.00001	0.29	<0.00005	<0.00001	<0.0003	<0.0001

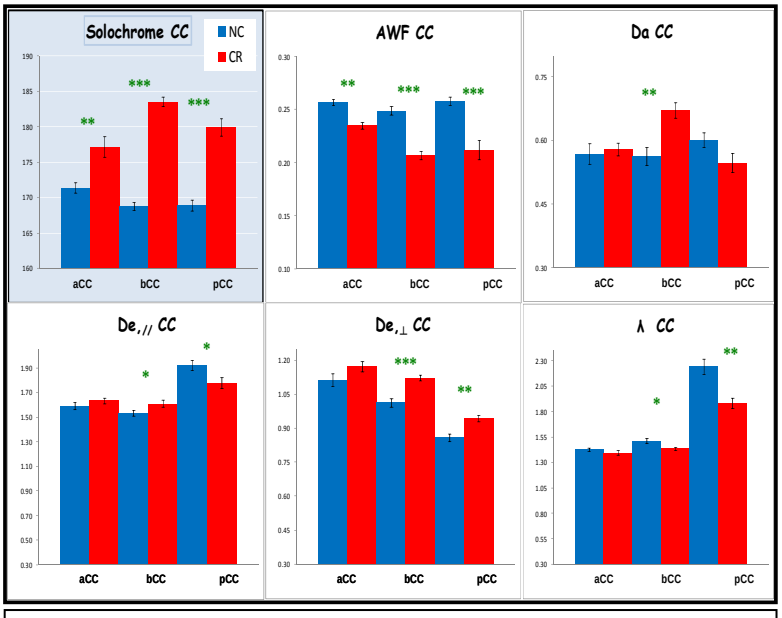

Figure1. ROI measurements of Solochrome and WMM metrics for cuprizone treated (CR) and control (NC) mice in the corpus callosum (rostral (aCC), middle (bCC), and caudal (pCC)); *p < 0.05, ** p< 0.005, *** p< 0.0001. Error bars are standard errors. Higher Solocrome mean intensity values = more demyelination. Diffusivities units: mm^2/ms: AWF and λ: dimensionless.

RESULTS: As expected, histological assessment showed intense demyelination in the CC of the CR mice, as indicated by higher pixel intensity values relative to NC mice (**Fig.1**), with the aCC being both the least affected area and that with greatest morphological heterogeneity**.** For DTI-derived diffusion metrics (FA, MD, $D_{//}$ and $D_{\perp}$), CR mice yielded results consistent with previous reports throughout the entire CC (i.e. reduced FA and increased MD and $D_{\perp}$ in comparison to NC mice, except for the FA in the aCC). Similarly, all kurtosis metrics in the CR mice showed significant decreases, except for $K_{//}$ in the aCC. For the WM model metrics, CR mice showed significant decreases in AWF in the entire CC, increased $D_{e,\perp}$ and decreased λ in bCC and pCC. (**Fig.1**). To investigate which metric would yield the strongest differentiation between the NC and CR, a mean effect size (Cohen's d) was calculated for each metric at each CC level. In the aCC, AWF (d = 2.6) and $K_{\perp}$ (d = 2.0) were the best differentiators. In the bCC, where demyelination is almost complete, MD, $\lambda_{\perp}$ and AWF (d = 4.1, 3.8, 3.7, respectively) were the best differentiators. In the pCC, $\lambda_{\perp}$, FA and AWF (d = 3.4, 2.4, 2.3, respectively) best differentiated the two groups.

DISCUSSION & CONCLUSION: Group differences of all of the diffusion metrics closely mirrored the distribution of demyelination as measured by the Solochrome, particularly in the bCC and pCC. These results help validate our WM model. In the CR group the WM metrics changed differently depending on the affected CC area; D_a was only increased at the bCC level, possibly reflecting the more intense axonal degeneration. $D_{e,\perp}$, $D_{e,||}$ and λ significantly changed in the bCC and pCC, which is consistent with an increase of water in the extra-axonal space. Our results also suggest that the AWF is sensitive to changes in the volume fraction of myelinated fibers, by being significantly decreased in the entire CC, mirroring the histological degree of demyelination and axonal atrophy. Finally, AWF and $K_{\perp}$ better captured the morphological heterogeneity seen at the aCC level, being the best group differentiators at this CC level. Overall, our results support the utility of DKI and the associated WM model metrics for characterizing changes associated with cuprizone-induced demyelination.

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