Pathological Correlates of the Decreased Axial Diffusivity in White Matter Injury

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Introduction

Decreased axial diffusivity ($\lambda_{||}$), derived by diffusion tensor imaging (DTI), has been shown to reflect axonal injury noninvasively. Demyelination, axonal injury, microglia/macrophage infiltration, astrogliosis, and edema may impact anisotropy of water diffusion in white matter tracts. There is no consensus on the pathology related cellular and structural events responsible for DTI parameter changes. Specifically, although $\lambda_{||}$ correlated with histological markers of axonal damage, the individual relationships of astrogliosis or microglia/macrophage infiltration with the changes in $\lambda_{||}$ has not yet been established. Therefore, the objective of the current study was to assess the relationship between the decreased $\lambda_{||}$ and histological markers including axonal injury, astrogliosis, and microglia/macrophage infiltration in the corpus callosum from cuprizone treated genetically altered mice with yellow fluorescent protein expressed by neurons.

Materials and Methods

Male Thy1-YFP-16 mice with background of C57BL/6 were fed 0.2% w/w of cuprizone. Age matched mice on a normal diet served as controls (N = 5). DTI data were acquired using a spin-echo diffusion weighted imaging sequence. Acquisition parameters were: TR = 1.5 s, TE = 50 ms, Δ = 25 ms, δ = 8 ms, NEX = 4, slice thickness = 0.5 mm, field-of-view = 3 cm^2, and data matrix = 256 $\times$ 256 (zero filled to 512 $\times$ 512). Diffusion sensitizing gradients were applied along six directions: [Gx,Gy,Gz] = [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [0,-1,1], and [1,0,-1]. Two b-values (0 and 0.768 ms/μm^2) were used. Subsequent histological experiments were performed using primary antibodies: Rat anti-CD11b for labeling microglia/macrophage and mouse anti-Glial Fibrillary Acidic Protein (GFAP) for labeling astrogliosis. Axonal damage and the extent of microglia/macrophage infiltration and astrogliosis were scored by blinded raters. Rank-based nonparametric tests, a Kruskal-Wallis test, and pairwise comparisons were used for statistic analysis. Significance level of 0.05 was used for the overall test. A Bonferroni correction was used for each set of 3 pairwise tests, giving a significance level of 0.05 / 3 = 0.0167.

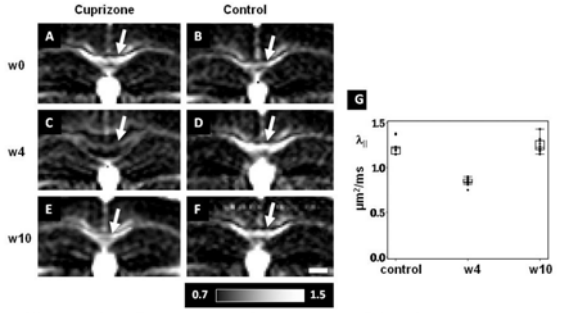

Figure 1. Decrease and recovery of $\lambda_{||}$ during cuprizone ingestion. N = 5. Scale bar: 500 µm.

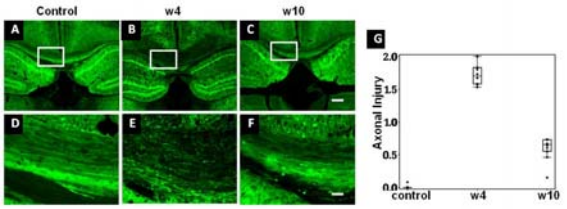

Figure 2. Reversible axonal injury during cuprizone ingestion. N = 5. Scale bar: 240 µm (A, B and C,); 50 µm (D, E and F).

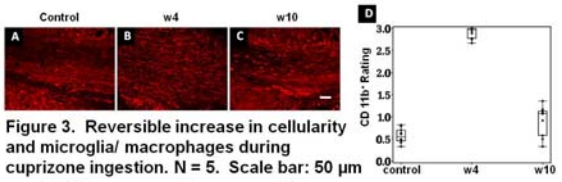

Figure 3. Reversible increase in cellularity and microglia/ macrophages during cuprizone ingestion. N = 5. Scale bar: 50 µm

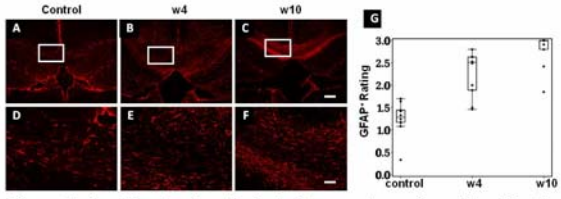

Figure 4. Sustained astrogliosis during cuprizone ingestion. N = 5. Scale bar: 240 µm (A, B and C); 50 µm (D, E and F).

Results

There was a reduction of $\lambda_{||}$ by 32% at 4 weeks compared with control (p = 0.0001) and a subsequent recovery from 4 to 10 weeks (p = 0.0002) despite continued cuprizone treatment. $\lambda_{||}$ at 10 weeks was not statistically significantly different from the control level (p = 0.40). Axonal injury level at 4 weeks was greater than control level (p < 0.0001) and greater than 10-week level (p = 0.0001). The YFP fluorescence recovered but did not return to control level at 10 weeks (p < 0.0001) suggesting that cuprizone-induced axonal injury was partially reversible, even with continued treatment. The level of infiltration of microglia/macro-phages was higher in week 4 specimens than that of the control (p = 0.0001). It is lower in week 10 than in week 4 specimens (p = 0.0001). There was a trend suggesting a difference between controls and week 10 specimens (p = 0.026, greater than the Bonferroni-adjusted significance level of 0.0167). Astrogliosis increased by 81% and 124% in the CC at 4 and 10 weeks, respectively, compared with the control. Cuprizone-induced astrogliosis was higher in 4 week specimens than in controls (p = 0.0005), and was higher in 10-week than in 4-week specimens (p = 0.0064).

Discussion

In the present study, Thy1-YFP-16 mice that express yellow fluorescent protein in neurons and axons were employed to examine the underlying pathological correlates of the decreased $\lambda_{||}$ in the CC after cuprizone treatment. As has been shown previously[1,2], $\lambda_{||}$ decreased at 4 weeks and recovered to the control level at 10 weeks of cuprizone treatment. This is in parallel to the observation that a significant YFP fluorescence loss was seen in the CC at 4 weeks and subsequently recovered at 10 weeks of cuprizone treatment. The current finding suggests that at least a significant portion of the axons remained viable and recovered during continuous cuprizone treatment. Our observation is consistent with the previous findings that decreased $\lambda_{||}$ indeed reflects the presence of axonal pathology.

Cells of the microglia/macrophage lineage increased significantly at 4 weeks and returned to the normal level at 10 weeks. In contrast, astrocyte activation increased at 4 weeks and continued to increase at 10 weeks. These data demonstrate that $\lambda_{||}$ correlates with axonal injury and increased numbers of microglia/macrophages, but not to astrogliosis. In summary, $\lambda_{||}$ is highly sensitive to axonal injury. Axonal injury induced by cuprizone is at least partially reversible. Decreased $\lambda_{||}$ reflects axonal injury, and the infiltration of microglia/macrophages, but does not correlate with astrogliosis. Despite the complexities of the cellular interactions, the fact that $\lambda_{||}$ parallels the axonal injury is an important step in detecting axonal damage non-invasively.

References **1**. Song et al., Neuroimage 2005; 26: 132-40. 2. Sun et al., Magn Reson Med, 2006; 55: 302-8.