Noninvasive Detection of Selective Vulnerability of Cerebral White Matter Tracts to Cuprizone

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Introduction

Selective cellular vulnerability to various insults is commonly seen in neurological diseases. For example, in Alzheimer's and Parkinson's disease, specific subpopulations of neurons are affected in lesions that are consistently observed across subjects. Interestingly, other neurons in the immediate vicinity retain their morphological integrity. However, selective axonal vulnerability of white matter has not received much attention, although white matter tracts of the central nervous system are anatomically and functionally heterogeneous. In white matter tracts, oligodendrocytes are a line of glial cells in the central nervous system that wrap axons with multilayered myelin for rapid impulse conduction. Cuprizone, a copper chelator, induces apoptosis of oligodendrocytes, leading to demyelination in the corpus callosum (CC) where axonal injury was recently reported[1,2]. Furthermore, demyelination and axonal injury in the CC showed a rostral-caudal pattern at 4 weeks of cuprizone ingestion[3,4]. Thus, previous studies implied that cerebral white matter tracts were impacted differently by cuprizone ingestion. Therefore, in the current study, axial diffusivity ($\lambda_{||}$) derived using diffusion tensor imaging (DTI) was used to detect vulnerable axons within white matter tracts during acute demyelination in mice fed cuprizone.

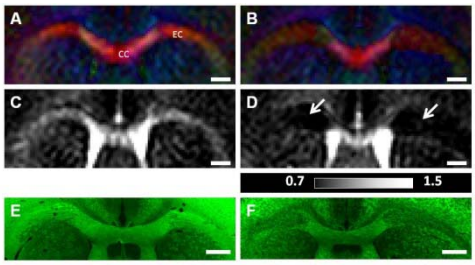

Figure 1. Color-coded RA maps (A and B), $\lambda_{||}$ maps (C and D) and histological sections (E and F) of the control (A, C and E) and cuprizone-fed (B, D and F) mouse brain. The scale bar: 500 µm (A-D), 512 µm (E and F).

Materials and Methods

Animal Model Male Thy1-YFP-16 mice on the C57BL/6 background were fed cuprizone-containing chow (0.2% w/w) for 4 weeks. Age-matched animals on a normal diet served as controls (N = 5).

Diffusion Tensor Imaging 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 × 256 (zero filled to 512 × 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. Data was expressed as mean ± standard deviation. Tissues were perfusion-fixed directly following DTI scanning for histological examinations.

Results

The current study examined the rostral region of corpus callosum (CC) and external capsule (EC) at the anatomical position 1 mm anterior to bregma. The CC and EC are readily visible in color-coded RA maps of control and cuprizone treated mouse brains with the color reflecting the primary axis of diffusion (red, left-right; green, superior-inferior; blue, anterior-posterior Figs. 1A and B). The EC (arrows, Fig. 1D) was hypointense in $\lambda_{||}$ maps of treated mouse brains, in contrast to the directly adjacent CC (Figs. 1C & D). Clear axonal injury in the EC of the treated mice is also demonstrated as the axonal beading and loss of YFP in the tissue sections (Fig. 1E and F).

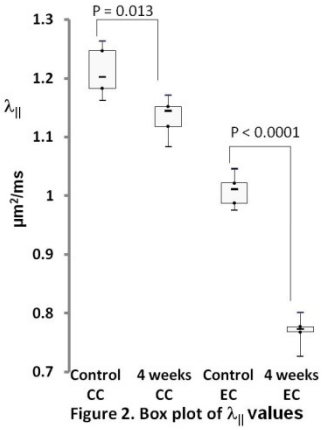

Figure 2. Box plot of $\lambda_{||}$ values

Both CC and EC were identified using the RA map. In control mice, $\lambda_{||}$ was 1.21 ± 0.04 and 1.01 ± 0.23 in the CC and EC respectively. In contrast, $\lambda_{||}$ was 1.13 ± 0.03 and 0.77 ± 0.03 in the CC and EC from the cuprizone treated mice. A 7% decrease in $\lambda_{||}$ was seen in CC from the treated mice compared with that of the control ($p = 0.013$); compared with a 24% decrease in EC $\lambda_{||}$ ($p < 0.0001$), suggesting more severe axonal injury in the EC induced by cuprizone (Fig. 2). More extensive axonal beading and loss of YFP, reflecting dysfunctioned axonal transport, in EC compared with the adjacent CC from the same brain was also seen under microscopic examinations (Fig. 3).

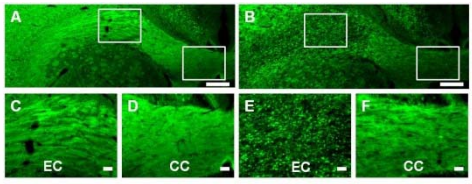

Figure 3. Microscopic photographs of the control (A, C and D) and cuprizone – fed (B, E and F) YFP mouse brains showing the rostral CC and EC. The scale bar: 215 µm (A and B), 38 µm (C-F).

Discussion

During acute demyelination induced by 0.2% of cuprizone, the caudal CC and dorsal hippocampal commissure are consistently demyelinated[3]. Subsequent studies reported axonal injury in the lesions with decreased $\lambda_{||}$ and immunostaining of dephosphorylated neurofilament and β-amyloid precursor protein[1,2]. In the current study, $\lambda_{||}$ maps had hypointense areas not only in the caudal CC and dorsal hippocampal commissure but also in the rostral EC. Axonal injury in the EC was manifested as significant axonal beading and YFP loss as seen in light microscopy. Thus, the rostral EC is selectively vulnerable to cuprizone. Loss of oligodendrocytes causes demyelination and axonal injury in the vulnerable tracts[5]. It still remains unknown why specific white matter tracts are particularly susceptible to cuprizone demyelination and/or axonal damage. In the current study, different extents of decreased $\lambda_{||}$ reflected the different degrees of axonal injury between the CC and EC during the acute demyelination induced by cuprizone. Our results suggested that decreased $\lambda_{||}$ was a sensitive biomarker for detecting selectively vulnerable white matter tracts non-invasively.

References

1. Song et al., Neuroimage 2005; 26: 132-40.
2. Sun et al., Magn Reson Med, 2006; 55: 302-8.
3. Stidworthy et al., Brain Pathol. 2003; 13:329-39
4. Wu et al., J Magn Reson Imaging, 2008; 27: 446-53.
5. Nave and Trapp, Annu. Rev. Neurosci. 2008; 31: 535-61