Noninvasive Detection of Axon and Myelin Damage in Mouse Corpus Callosum

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Introduction

A reproducible time course of oligodendrocyte loss and subsequent demyelination in the corpus callosum (CC) is observed after feeding 0.2% cuprizone to 8-week old C57BL/6 male mice. Demyelination is extensive after several weeks of dietary cuprizone, yet can be reversed after returning the mice to normal chow. Axonal injury may also be associated with disease progression in this cuprizone model (1,2). Previously, axial ($\lambda_{\parallel}$) and radial ($\lambda_{\perp}$) diffusivities derived from diffusion tensor imaging (DTI) have been proposed and characterized as surrogate markers of the axonal damage and demyelination in mouse brain white matter (3). In the present study, the time course of both axonal injury and demyelination in CC was examined in mice throughout cuprizone intoxication and recovery.

Materials and Methods

Animal preparation

The control and experimental group each consists of six eight-week old male C57BL/6 mice. For the experimental group, mice were placed on a diet of 0.2% cuprizone thoroughly mixed into milled chow for twelve weeks. After twelve weeks of feeding, mice were placed on a normal diet (chow with no cuprizone) for another twelve weeks. For the control group, mice were placed on a normal diet for twenty-four weeks.

Diffusion Tensor Imaging

All mice underwent biweekly DTI examinations starting prior to the initiation of cuprizone feeding. For acquisition of DTI, a conventional spin-echo imaging sequence modified by adding the Stejskal-Tanner diffusion sensitizing gradient pair was employed. The imaging parameters include TR 1.5 sec, TE 50 msec, Δ 25 msec, δ 10 msec, NEX 4, slice thickness 0.5 mm, field-of-view 3 cm, 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 diffusion sensitizing factors or b-values (0 and 0.768 ms/μm^2) were used. Three quantitative indices including RA, axial diffusivity ($\lambda_{\parallel} = \lambda_1$), and radial diffusivity ($\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$) were measured in the corpus callosum.

Histological Examination

Myelin integrity was evaluated using Luxol fast blue stain (LFB) (Sigma, Saint Louis, MO). Immunohistochemical examination of axonal damage was evaluated using primary antibodies: β-amyloid precursor protein (β-APP) (1:100; Zymed Laboratories, Inc., South San Francisco, CA) and non-phosphorylated neurofilament (np-NF) (SMI-32, 1:100; Sternberger Monoclonals, Lutherville, Maryland). The binding of the primary antibodies was revealed by avidin-biotion-peroxidase method with reagents supplied by Zymed Laboratories. Negative controls omitting the primary antibody were used for each case.

Results

The temporal evolution of both $\lambda_{\parallel}$ and $\lambda_{\perp}$ is summarized in Fig. 1. At 4 weeks after cuprizone treatment, $\lambda_{\parallel}$ significantly decreased by 28 % in CC. This decreased $\lambda_{\parallel}$ retuned to control level two weeks later. There was no detectable change in $\lambda_{\perp}$ during the first 4 weeks of cuprizone feeding. However, it gradually increased from the control level at 6 to 8 weeks and reached the plateau between 8 to 12 weeks after cuprizone feeding. Comparing to age-matched control mice, at 6, 8, 10, and 12 weeks, $\lambda_{\perp}$ increased 27% ($p = 0.07$), 49% ($p < 0.05$), 75% ($p < 0.05$), and 66% ($p < 0.05$). In the recovery phase, $\lambda_{\perp}$ values gradually returned toward the normal levels. The normalization of $\lambda_{\perp}$ was not complete at the end of the study.

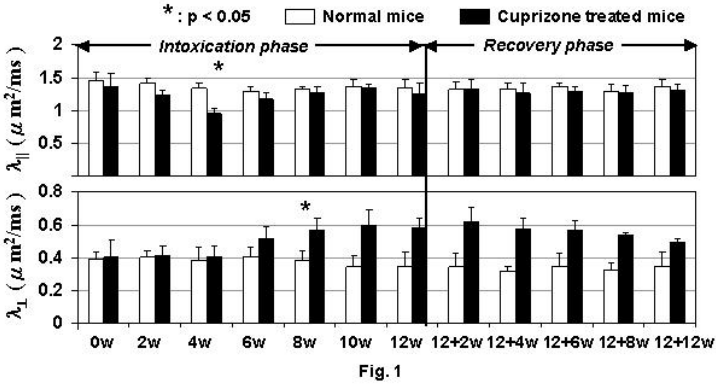

Fig. 1

Representative results of the histological examinations of CC are shown in Fig. 2. Demyelination was clearly demonstrated by the negative stains of Luxol fast blue (LFB) at 4 and 12 weeks after cuprizone feeding. The remyelination was evident by the positive stain of LFB at 12+12 weeks. These findings are consistent with those predicted by the time course of $\lambda_{\perp}$ in Fig. 1 with the exception that the demyelination was not detected until 6 weeks by $\lambda_{\perp}$. The intense stains of both β-APP and np-NF are detected at 4 weeks after cuprizone feeding. This is in agreement with the prediction of decreased $\lambda_{\parallel}$ as shown in Fig. 1.

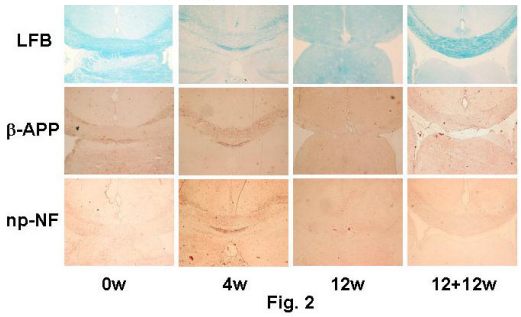

Fig. 2

Discussions

The feasibility of using $\lambda_{\parallel}$ and $\lambda_{\perp}$ to detect axonal damage and demyelination was demonstrated in this study. The modulation of $\lambda_{\perp}$ in the course of cuprizone treatment is consistent with LFB stains in CC supporting the proposed use of increased $\lambda_{\perp}$ as demyelination marker. A less frequently reported axonal injury in curpizone treated mice, has also been detected by the decreased $\lambda_{\parallel}$ at 4 weeks of treatment and was validated by histology. Our current finding agrees with previous report that $\lambda_{\parallel}$ and $\lambda_{\perp}$ may serve as surrogate markers of axonal injury and demyelination in mouse CNS white matter.

References

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