Axial Diffusivity in Optic Nerve Correlates Retinal Ganglia Cell Loss in EAE Mice

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Introduction

Although MS has been considered an inflammatory demyelindating disease, increasing evidences have supported that the axonal and neuronal injury are main causes for the permanent disability in patients. However, the relation between axonal and neuronal damage is still not clear in MS. Hypothetically, the axonal damage may induce dying-back degeneration leading to the death to the neuronal cell bodies. Using MRI, axial ($\lambda_{\parallel}$) and radial ($\lambda_{\perp}$) diffusivities derived from diffusion tensor imaging (DTI) have been demonstrated as potential biomarkers to detect axonal and myelin damage (1, 2). Recent studies have shown the feasibilities of $\lambda_{\parallel}$ and $\lambda_{\perp}$ to identify the damage to optic nerve of mice affected by experimental autoimmune encephalomyelitis (EAE), an animal model for human MS (3). To better understand the relation of injuries present in neurons and axons, in this study, the retinal ganglia cell (RGC, the cell body of axons of optic nerve and tract) was evaluated by hematoxylin and eosin (H&E) stain. The density of RGC was quantified from EAE and control mice. The correlation between RGC and the in vivo measurements of $\lambda_{\parallel}$ and $\lambda_{\perp}$ from optic nerves was evaluated.

Materials and Methods

Six 8-week-old female C57BL/6 mice were immunized with myelin oligodendrocyte glycoprotein (MOG) amino acids 35-55 emulsified in complete Freund's adjuvant (CFA). Pertussis toxin (PTX) was injected intravenously on the day of immunization and three days later. At 3 months after immunization, when the mice were chronically affected, mice were examined using DTI. Five age-matched female C57BL/6 mice served as controls. For acquisition of *in vivo* DTI, a conventional spin-echo imaging sequence modified by adding the Stejskal-Tanner diffusion sensitizing gradient pair was employed. The imaging parameters were 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 with b-values of 0 and 0.85 ms/μm^2. 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 optic nerve (ON). At the conclusion of *in vivo* DTI, mice were perfusion fixed with 10% formalin. The eye was carefully dissected and embedded in paraffin. Three-µm-thick slices were collected from the mid-sagittal section of the eye. The tissue sections were stained by H&E. Sections were examined with a Nikon Eclipse 80i microscope equipped with a 20× objective and captured by a Photometrics CCD digital camera with MetaMorph image acquisition software (Universal Imaging Corporation, Downington, PA). The RGC were counted through the whole retinal ganglia cell layer in each section.

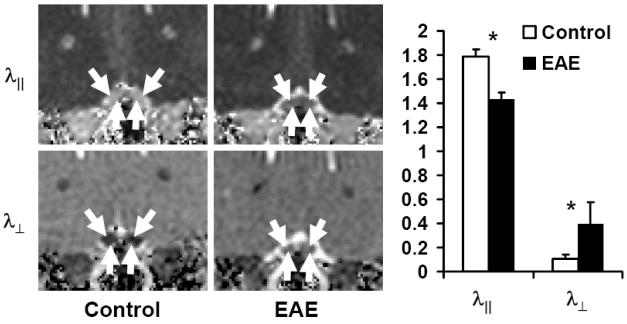

Fig. 1 DTI of EAE and Control Optic Nerve

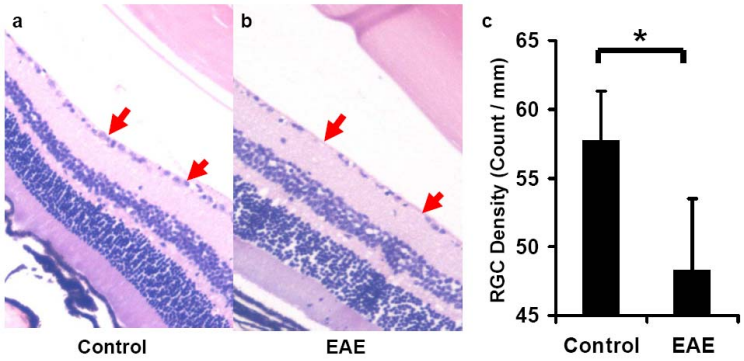

Fig. 2 Histology of Retina from Control and EAE Mice

Results Comparing to the control optic nerve, a 20% decrease in $\lambda_{\parallel}$ and 270% increase in $\lambda_{\perp}$ were measured from EAE optic nerve suggestive of severe axonal and myelin damage (Fig. 1, where arrows indicate the optic nerves). A significant 16% loss of RGC from EAE mice was also observed by microscopic examination on the H&E stained slides (Fig. 2, where red arrows indicate RGC layers). Significant correlations were observed between RGC density vs. $\lambda_{\parallel}$ and $\lambda_{\perp}$ respectively (Fig. 3).

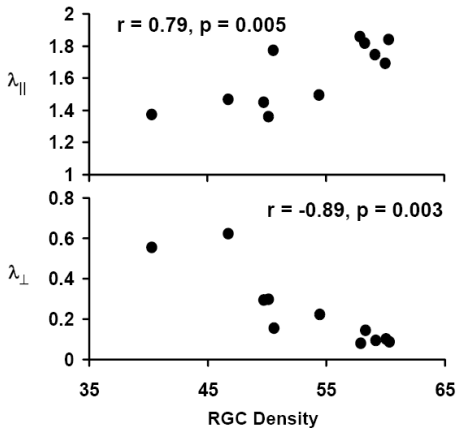

Fig. 3 Correlation of RGC vs. $\lambda_{\parallel}$ and $\lambda_{\perp}$

Discussions and Conclusions

In this study, significant correlations between RGC loss and DTI abnormalities to optic nerve were demonstrated in EAE mice. Both $\lambda_{\parallel}$ and $\lambda_{\perp}$ in optic nerve showed significant correlations to RGC density, suggesting a possible causal relationship between the axonal damage and the RGC loss. Although it is unclear whether it is the Wallerian or dying-back degeneration between the RGC and optic nerve damages, further studies may be designed to determine the sequence of events of the observed axonal and neuronal damages. The gained knowledge in this pathological process may provide a clearer target for therapeutic intervention to prevent axons or neurons from the secondary damage improving the efficacy of the treatment.

References

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(3) Sun et al., Neurobiology of Disease, 2007; 28: 30-38.

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