Evolving axon degeneration in optic nerve crush mice assessed using in vivo diffusion tensor imaging

P. Sun[1], X. Zhang[1], Q. Wang[2], and S-K. Song[1]
[1]Radiology, Washington University in St. Louis, Saint Louis, Missour, United States, [2]Mechanical Engineering and Materials Science, Washington University in St. Louis

Introduction

Glaucoma is a leading cause of blindness worldwide. It is an optic neuropathy originating from intraocular pressure induced optic nerve damage and the ensuing retrograde degeneration of retinal ganglion cells and the progressive loss of vision. Diffusion tensor imaging (DTI) is a widely used imaging technique that reflects integrity and pathology of the central nervous system (CNS) tissues. In this study, we applied in vivo DTI to investigate the relationship between optic nerve injury and retinal ganglion cell damage using a widely accepted mouse model of optic nerve crush. In vivo DTI findings were validated using matched immunohistochemistry on fixed tissues

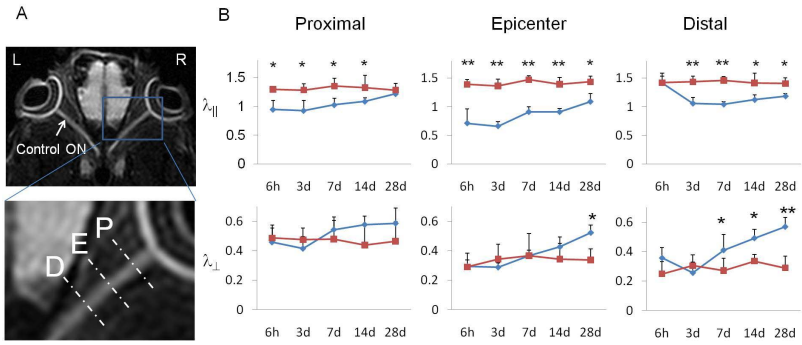

Figure 1 The dotted lines marked the center of proximal (P), epicenter (E) and distal (D) segments of the ROI (A); Time course of DTI parameters measured from longitudinal analysis of ONC injured optic nerves (blue line) and control optic nerves (red line)(B). $\lambda_{\parallel}$ and $\lambda_{\perp}$ expressed as mean± standard deviation (n=5) are shown at each segment. Statistical differences indicated in relation to control group:*P<0.05, **P<0.01.

Material and Method:

Animal Model: Adult female C57BL/6 mice (n = 20) at 8-12 weeks of age were employed. The optic nerve was surgically exposed by blunt dissection under an operating microscope. The right nerve was crushed approximately 1.0 mm behind the globe for 20 seconds and the contralateral optic nerve served as the control.

MRI: In vivo DTI performed at 6 hr, 3, 7, 14, and 28 days after unilateral optic nerve crush to determine the time course of axonal and myelin injury. Mice were divided into two groups: Group1 (n = 5) undergoing longitudinal DTI and Group2 (n = 15, 3 per time point) undergoing cross-sectional DTI measurements followed by histological analysis. Retrograde retinal ganglion cell labeling was performed one week before ONC to correlate the retinal ganglion cell loss with the severity of optic nerve injury. A spin-echo sequence incorporating a pair of diffusion sensitizing gradients was used for DWI with the following parameters: TR, 1.5 s; TE, 35 ms; Δ, 25 ms; δ, 8 ms; b-value, 0 and 849 s/mm^2; Image resolution: 156 × 156 × 400 μm^3; total acquisition time: 2 hours.

Histology: The optic nerves were isolated, fixed using 4% paraformaldehyde, embedded in paraffin, sectioned at five-μm-thick, and stained with antibodies against axons (SMI-31: phosphorylated neurofilament) and myelin (MBP: myelin basic protein). Retinas were dissected and flat-mounted on glass slides for FluoroGold labeled RGC counting to correlate the retinal ganglion cell injury with the severity of optic nerve injury.

Data Analysis: DTI maps were calculated from the diffusion weighted images. A line-shape ROI of optic nerve with one voxel thick was defined using ImageJ (Wayne Rasband, NIH, USA). The dotted lines in Fig. 1A marked the center of proximal (P), epicenter (E) and distal (D) segments of the optic nerve, where both DTI data analysis and histology were performed for matched validation.

Results

Reduction of axial diffusivity at all sites suggested axonal damage beginning at 6 hours lasting 28 days post optic nerve crush (Figs. 1B). Both epicenter and distal site showed significant increase in radial diffusivity at 7 - 28 days suggestive of myelin damage. This MRI finding correlated with the loss of intact axon counts (Figs. 2A-B). MBP counts confirmed the myelin damage occurred at the same time period. The correlation between retinal ganglion cell and axon counts suggests the damage of optic nerve axon may be the initiation site to RGC degeneration of this model (Fig. 3).

Conclusion

In conclusion, the current results suggest that DTI may be used to identify axon injury and demyelination tracking the disease progression of glaucoma in vivo.

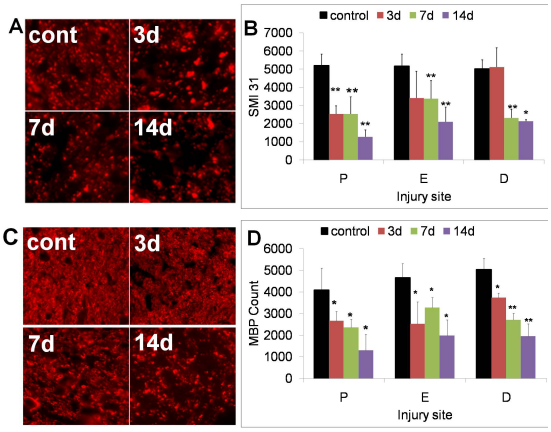

Figure 2 Immunohistochemistry of SMI31(A) and MBP(C) of optic nerve cross-sections at proximal site from control and ONC injured mice at 3, 7 and 14 days. SMI31 (B) and MBP positive axon counts (D) in the optic nerves at proximal, epicenter and distal segment show different patterns of injury evolution. *P<0.05, **P<0.01. 60× magnification.

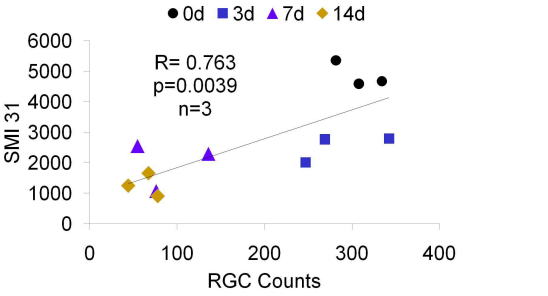

Figure 3 Correlation between the retinal ganglion cell and intact axon (SMI31-positive) counts in a subgroup of experimental animals. The scatter plot shows a correlation between FG-labeled RGC counts and the number of positive SMI31 stained axons in proximal site (R = 0.76; P = 0.004). Solid line drawn through data-points is the best fit.