In Vivo Assessment of Optic Nerve Degeneration in Glaucoma Rat Model using Diffusion Tensor Imaging

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Introduction

Glaucoma is a neurodegenerative disease characterized by visual field loss, cupping of the optic nerve head, and irreversible loss of retinal ganglion cells, and thus their axons [1]. Increase in intraocular pressure (IOP) is believed to be the major risk factor for glaucoma though the disease mechanism is not fully understood yet [1]. A recent study implicated the neural degeneration in intracranial optic nerve, lateral geniculate nucleus and visual cortex in this disease [2]. Clinical diagnosis of glaucoma relies on IOP measurement using tonometer, visual field test and ophthalmoscopy. In this study, we hypothesized that diffusion tensor imaging (DTI) can detect and characterize optic nerve (ON) degeneration during glaucoma disease progression in established animal models. In particular, changes of directional diffusivities may be associated with the gradual loss of axonal density at the ON of the glaucomatous eye. This approach could be valuable for potential MRI assessment of ON integrity in glaucoma patients, and understanding the pathophysiological cascades involved in this disease.

Materials and Methods

Glaucoma Induction: Experimental glaucoma was performed in seven adult female Sprague-Dawley rats (280-300g, N=7) with the detailed procedures described in Ref 3. Briefly, the right eye of each animal was induced with glaucoma by elevating the IOP whereas the left eye served as the control. The episcleral and limbal veins of the right eye were photocoagulated using an Argon laser (Ultima 2000SE Argon Laser, Coherent, USA). A second laser treatment in the same setting was applied 7 days later. This procedure consistently elevated the IOP in the right eye about 1.5 times above normal, which was confirmed by using a Tonopen XL Tonometer (Mentor Massachusetts, The Netherlands).

Diffusion Tensor Imaging: *In vivo* DTI was performed at 8, 10, 12, 14 and 21 days after the first laser treatment. All experiments were performed using a 7-T Bruker scanner (70/16 Bruker PharmaScan, Germany). Diffusion weighted (DW) images were acquired with a respiration-gated spin echo 8-shot EPI readout sequence. An encoding scheme of 30 gradient directions which are homogenously distributed on the unit sphere was used to acquire DW images [4]. The imaging parameters were: TR/TE = 3000/36.5ms, Δ=15ms, δ=5ms, slice thickness = 1mm, FOV = 35mm, data matrix = 256 x 256 (zero filled to 512 x 512), image resolution = 136 x 136 μm^2 and two b-values were used (0 and 1000 s/mm^2). The sequence was repeated four times for signal averaging, resulting in an acquisition time of approximately 90 minutes depending on the respiratory rate. The diffusion tensors were extracted and diagonalized using an in-house Matlab program interfaced to a software for CNLS estimation for DTI (by Dr. CG Koay, STBB/LIMB/NICHD, NIH). Axial diffusivity ($\lambda_{//}$), radial diffusivity ($\lambda_{\perp}$) and fractional anisotropy (FA) were measured in the left and right ON with the identical ROI in all time points. ROI was drawn based on $\lambda_{//}$, $\lambda_{\perp}$ and FA map to avoid covering any CSF. All measurements on the left and right ON were compared using paired t-test, and $p < 0.05$ was considered as significant.

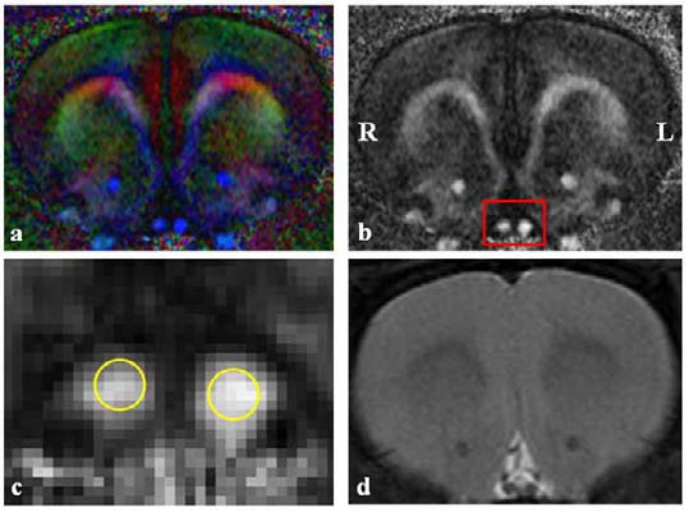

Figure 1. **(a)** Typical color and **(b)** FA map of a glaucoma-induced rat at 21 days after the first laser treatment. **(c)** shows an enlarged view of the optic nerves (ONs) in (b) indicating the obvious difference between the right glaucomatous and left normal ON. ROI was also drawn in (c) to demonstrate the placement for the left and right ON. **(d)** T2-weighted image. R: right, L: left

Results and Discussions

The sequence was tested using a water phantom to demonstrate the general accuracy. At 20^{o}C, the measured apparent diffusion coefficient of water was within 5% error of the value in literature [5] and the water diffusion was isotropic as expected. Fig. 1 shows the typical color, FA map and T2-weighted image of a glaucomatous rat at 21 days after the first laser treatment illustrating the appreciable difference between the right injured and left control ON. As shown in Fig. 2, statistically significant differences were observed between the $\lambda_{\perp}$ and FA of the right and left ON at all time points. However, there was no significant difference for $\lambda_{//}$. $\lambda_{\perp}$ and FA showed increasing and decreasing trend respectively from between day 8 and day 21 after first laser treatment. Given that loss of axons in the ON was observed in previous studies [6], the insignificant $\lambda_{//}$ deviation in the glaucomatous ON suggested that the remaining axons were still largely intact. Furthermore, given that the axonal loss was approximately 19% at 21 days after first laser [6], the corresponding $\lambda_{\perp}$ increase observed here (28%) was likely caused by the axonal density decrease instead of demyelination. Histological analyses are currently under way to validate these *in vivo* DTI observations in the animals studied.

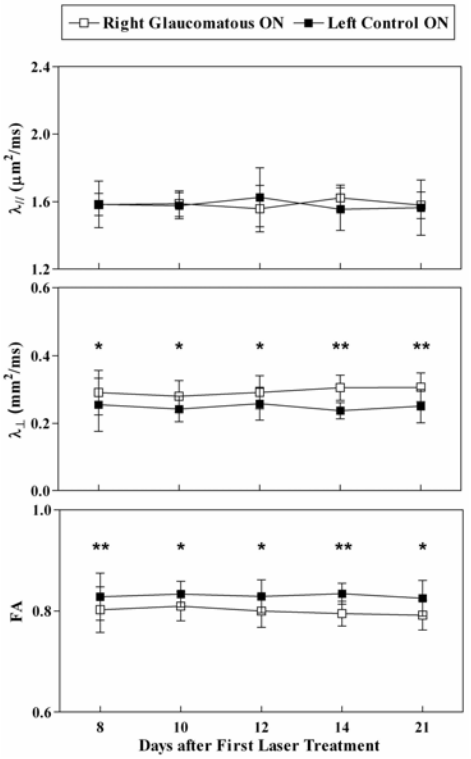

Figure 2. Changes in different DTI parameters, FA, $\lambda_{//}$ and $\lambda_{\perp}$ with respect to time. There is significant difference between left and right ON in all time points for FA and $\lambda_{\perp}$ (N=7). * $p < 0.05$, ** $p < 0.01$

Conclusions

Despite of the high prevalence of the disease, the current clinical measures of function and structure of the visual pathway might not be good surrogates of the neuronal integrity [1]. The present experimental results for the first time indicated that *in vivo* DTI holds promise for diagnosing and assessing glaucoma progression as well as treatment effect. The information obtained from DTI may serve as valuable and reliable indices for evaluating the neuronal integrity and hence the visual pathway.

References

[1] Artes PH et al., Prog. Retin. Eye Res. 2005; 24: 333-54.
[2] Gupta N et al., Br. J. Ophthalmol. 2006; 90: 674-8.
[3] Ji JZ et al., Eur. J. Neurosci. 2004; 19: 265-72.
[4] Jones D et al., Magn. Reson. Med. 1999; 42: 515-525.
[5] Le Bihan D et al., Radiology 1998; 168: 497-505.
[6] Levkovitch-Verbin H, Eye 2004; 18: 1066-1074.