Morphological and Microstructural Changes in the Eye and the Brain in an Experimental Glaucoma Model Induced by Crosslinking Hydrogel Injection

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Target Audience: Basic and clinical scientists with interests in how *in vivo* anatomical MRI, magic angle-enhanced MRI (MA-MRI) and diffusion tensor imaging (DTI) can help determine the structural integrity and pathophysiology of the eye and brain's visual system in glaucoma.

Purpose: Glaucoma is the second leading cause of blindness in the world and is an irreversible neurodegenerative disease of the visual system [1]. While experimental glaucoma models are important to the study of the disease mechanisms and potential treatment strategies, limited models have been available to provide sustained intraocular pressure (IOP) elevation while keeping a clear visual axis for normal visual input to the eye. Recently, a novel glaucoma mouse model has been developed based on a crosslinking hydrogel that gives sustained IOP elevation and a transparent medium after intracameral injection, and the longitudinal profile of retinal ganglion cell loss was successfully demonstrated with *in vivo* optical imaging [2]. In this study, we further characterized this model in rats using *in vivo* anatomical MRI, MA-MRI and DTI with an aim to determine the morphological and microstructural changes in the whole eye and the brain in experimental glaucoma, and to provide complementary information to the pathophysiological events observed in optical imaging studies.

Methods: Seven adult Long Evans rats were injected intracamerally to the right eye with 20μL of a mixture of 4% vinysulfonated hyaluronic acid and 4% thiolated hyaluronic acid dissolved in PBS, which formed into a solidified, optically clear hydrogel in about 3 minutes [2]. The left eye was untreated and served as an internal control. IOP was measured in both eyes using the TonoLab rebound tonometer for 2 weeks after hydrogel injection. DTI was performed at 3 (n=7), 7 (n=7) and 14 (n=3) days after hydrogel injection under isoflurane anaesthesia, while MA-MRI was performed at 11 days (n=5) after hydrogel injection under ketamine/xylazine anesthesia to minimize involuntary eye movement. Anatomical T2-weighted imaging (T2WI) was taken from non-diffusion-weighted b_o images in DTI. All scans were performed using a 9.4-Tesla/31-cm Varian/Agilent scanner with a volume transmit and surface receive coil. MA-MRI was acquired using the spin-echo sequence with TR/TE=1000/13.6ms, in-plane resolution=55x55μm^2 and slice thickness=1.0mm. DTI was acquired using a fast spin-echo sequence with 12 diffusion gradient directions at b=1000s/mm^2 and 2 non-diffusion-weighted b_0 images, TR/TE=2300/27.8ms, ETL = 8, δ/Δ=5/17ms, NEX=4, acquisition resolution=135x135μm^2 and slice thickness=1mm. Regions of interest (ROIs) were manually drawn bilaterally on the anterior chamber and vitreous to measure the cross-sectional areas on the b_0 images from DTI (yellow borders in Fig. 2a). For MA-MRI, signal intensity near the magic angle in the sclera was measured (red arrows in Fig. 2d). Fractional anisotropy (FA), axial diffusivity ($\lambda_{//}$), radial diffusivity ($\lambda_{\perp}$) and mean diffusivity (MD) maps were computed using DTIStudio. ROIs were manually drawn on the prechiasmatic optic nerves and optic tracts based on FA, $\lambda_{//}$ and $\lambda_{\perp}$ maps and the rat brain atlas.

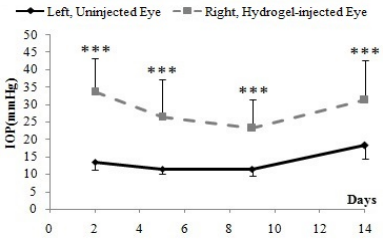

Figure 1. IOP profiles of the hydrogel-induced experimental glaucoma model from 2 days to 2 weeks after hydrogel injection. (Two-tailed paired t-tests between left and right eyes: ***p<0.001)

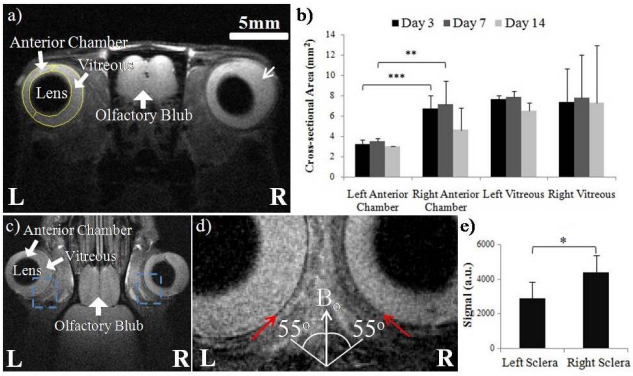

Figure 2. (a) Representative T2-weighted image of the rat eyes showing anterior chamber enlargement (open arrow) after hydrogel injection to the right eye; (b) Cross-sectional areas of the anterior chamber and the vitreous in the left normotensive eye and right hypertensive eye across time. (c) Sample MA-MRI image; (d) Enlarged view of the rat eyes from the blue dashed boxes in (c). (e) Signal intensities of the sclera near the magic angle at about 55° to main magnetic field (Bo) [red arrows in (d)]. (Two-tailed paired t-tests between left and right eyes: *p<0.05, **p<0.01, ***p<0.001)

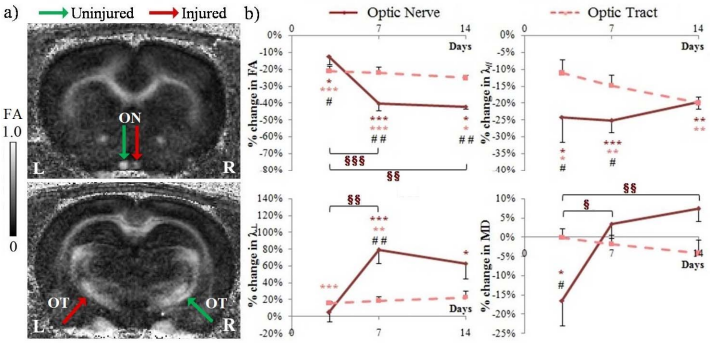

Figure 3. (a) Representative fractional anisotropy (FA) maps of the optic nerve (top) and optic tract (bottom) at 7 days after hydrogel injection to the right eye. (b) Spatiotemporal changes in DTI quantitation (mean ± standard error) in the injured optic nerve and injured optic tract projected from the hypertensive right eye relative to the uninjured visual pathways at 3, 7 and 14 days after hydrogel injection. (Two-tailed paired t-tests (i) between injured and uninjured eyes: *p<0.05, **p<0.01, ***p<0.001 (ii) between optic nerve and optic tract: #p<0.05, ##p<0.01; Post-hoc Tukey's tests between days 3,7 and 14: §p<0.05, §§p<0.01, §§§p<0.001)

Results: Upon intracameral hydrogel injection to the right eye, IOP elevated and sustained in the right eye up to the experimental period of 2 weeks (Fig. 1). The right anterior chamber significantly enlarged within the first week with no significant change in the vitreous (Figs. 2a-b). In addition, the sclera of the hypertensive right eye showed significantly higher T2-weighted signal intensity than the normotensive left eye near the magic angle (Figs. 2c-e). Along the visual pathways in the brain, significant decrease in FA and $\lambda_{//}$ was observed in the optic nerve and optic tract projected from the hypertensive eye throughout the experimental period (Fig. 3). The highest rates of FA and $\lambda_{//}$ decrease in the injured optic nerve occurred within the first 7 and 3 days respectively after hydrogel injection, whereas a delayed $\lambda_{\perp}$ increase occurred in the injured optic nerve between 3 days and 1 week after hydrogel injection. Differences in DTI measurements between injured and uninjured optic tracts were fairly constant and generally smaller than the optic nerves during the experimental period.

Discussion and Conclusions: A novel, hydrogel-induced experimental glaucoma model was established in rats. Within the eyeball, although there was no significant change in vitreous size, MA-MRI detected for the first time *in vivo* the significantly higher T2-weighted signal intensity in the sclera of the hypertensive eye compared to the normotensive eye. This suggested the microstructural reorganization in the fibrous tissues as demonstrated in our recent *ex vivo* ocular MA-MRI study [3]. Within the brain's visual system, our DTI findings of the early $\lambda_{//}$ decrease and delayed $\lambda_{\perp}$ increase in the optic nerve may reflect different progression rates for several neurodegenerative events known to occur along the neural pathways such as early axonal injury and delayed demyelination [4]. In addition, the temporal profile of overall microstructural integrity (as reflected by FA) in the optic nerve appeared to coincide with the temporal profile of retinal ganglion cell loss in a recent optical imaging study using the same hydrogel-induced model but in mice [2]. Because of the clear optical medium of the hydrogel, the current experimental glaucoma model may provide a platform to investigate the eye-brain-behavior relationships, the pathophysiological events in the whole visual system and neuroprotective strategies to both the eye and the brain in glaucoma using combined MRI, optical imaging and visual behavioral testing in future studies.

References: [1] Kingman S., et al. Bull World Health Organ. 2004; [2] Leung C.K., ARVO: 2087, 2014; [3] Ho L.C. IOVS, 2014; [4] Sun S.W., Neuroimage, 2008.