In vivo evaluation of ocular physiology and structural integrity of the optic nerve upon whole eye transplantation using gadolinium-enhanced MRI and diffusion tensor imaging

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Target Audience: Basic and clinical researchers with interests in how dynamic contrast-enhanced ocular MRI and diffusion tensor MRI (DTI) of the visual pathway can help establish a viable orthotopic model of vascularized whole eye transplantation (WET) in the rat.

Purpose: Approximately 39 million people in the world suffer from complete blindness [1] mostly due to the inability of retinal ganglion cells to regenerate. WET gives the opportunity to provide viable retinal ganglion cells and the entire optical system to recipients with vision loss and irreversible injury to the eye. In order to promote normal functioning of the eye, proper ocular circulation is necessary following WET. A functional face transplant protocol has previously been established by our group [2] and has been recently expanded to include the whole eye, optic nerve and ophthalmic blood supply. The purpose of this study is to demonstrate the viability of this orthotopic WET model by assessing the aqueous humor dynamics and tissue permeability of the transplanted eye using gadolinium (Gd)-enhanced MRI [3-4]. In addition, DTI was employed to determine the structural integrity of the optic nerve following WET.

Methods: ***Animal Preparation:*** Five Lewis rats received WET on the right eye only (Fig. 1). The donor flap contained all ocular tissues anterior to the optic chiasm, a section of temporal bone, and the skin of the eye lid and external ear. In the recipient site, a similar region of skin tissue and the eye socket content were removed, with the optic nerve cut at the base of the globe. The graft was transplanted and anastomoses between carotid arteries and external jugular veins were performed. Intraocular pressure (IOP) was measured before MRI experiments using the TonoLab rebound tonometer. Gd-DTPA (Magnevist) was intraperitoneally (i.p.) injected at a dose of 0.3mmol/kg after one T1-weighted image (T1WI) at baseline was acquired. ***MRI Protocols:*** Four animals were scanned at 3 weeks after WET, and 1 animal at 10 weeks after WET. All scans were performed using a 9.4-Tesla/31-cm Varian/Agilent scanner, with a transmit-receive volume coil for Gd-MRI, and volume transmit and surface receive coil for DTI. For Gd-MRI, T1WIs at 30s-temporal resolution were continuously acquired for 1.5 hours using a fast spin echo sequence. The imaging parameters were: TR/TE=600/8ms, ETL=8, in-plane resolution =135x135μm^2, slice thickness=1mm. Slices were oriented to bisect the center of the eyes. DTI was acquired before Gd-MRI using a fast spin echo sequence, with 12 diffusion gradient directions at b=1.0ms/μm^2 and two b=0ms/μm^2. Other imaging parameters included: TR/TE= 2300/27.8ms, ETL=8, δ/Δ=5/17ms, acquisition resolution=135x135μm^2, and slice thickness=0.5mm. Slices were oriented orthogonal to the prechiasmatic optic nerves. ***Data Analysis:*** For Gd-MRI, regions of interest (ROIs) were drawn manually on the anterior chamber (AC) and vitreous of both eyes in T1WI. The Gd-signal time course from each ROI measurement was analyzed. The initial rate of Gd increase, peak % Gd signal enhancement and time-to-peak were calculated and compared between both eyes. DTI paramatric maps including fractional anisotropy (FA), axial diffusivity ($\lambda_{//}$) and radial diffusivity ($\lambda_{\perp}$) were computed using DTIStudio. ROIs were drawn on prechiasmatic optic nerves and compared bilaterally.

Fig. 1: WET graft (A and B). ON: optic nerve; TB: temporal bone; TN: trigeminal nerve; ELG: external lacrimal gland; CCA: common carotid artery; EJV: external jugular vein. Recipient immediately after WET (C). Note the color change in the eye and ear indicative of restoration of blood circulation. Inset shows apposition of optic nerve.

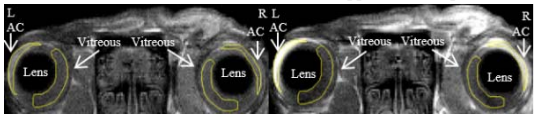

Fig. 2: T1-weighted images at 0-10min (left) and 60-70 min (right) after systemic Gd administration, showing rat ocular anatomy of anterior chamber (AC), vitreous and lens 3 weeks after WET.

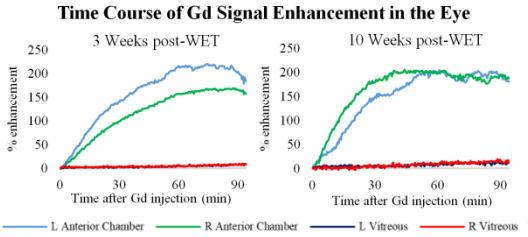

Fig. 3: Average time courses of % Gd enhancement in anterior chamber and vitreous of both eyes at 3 and 10 weeks after WET.

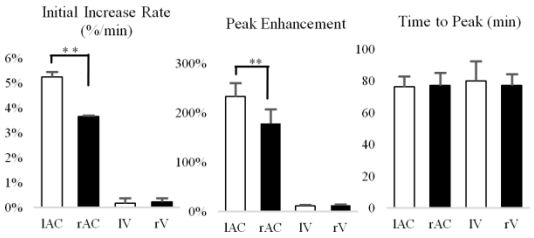

Fig. 4: Quantitative comparisons of initial rate of Gd increase, peak % Gd signal enhancement, and time to peak in the anterior chamber (AC) and vitreous (V) of uninjured left (l) eye and transplanted right (r) eye at 3 weeks after WET. (Two-tailed paired t-tests: **p<0.01)

Results: IOPs of uninjured left eye and transplanted right eye were 15.9±3.1 mmHg and 16.5±3.2 mmHg respectively before MRI experiments. Fig. 2 shows the rat eye anatomy and Gd enhancement in the AC across time. Time courses of Gd signal enhancement in AC and vitreous are shown in Fig. 3. In Figs. 3 and 4, the right AC at 3 weeks after WET had a similar time to peak, but a significantly lower peak intensity and lower initial increase rate than left AC. For the rat at 10 weeks after WET (Fig. 3), the right AC had a comparable peak intensity to the left AC, however the right AC had a shorter time to peak. Limited Gd enhancement was observed in the vitreous with no significant difference between left and right eyes (two-tailed paired t-tests, p>0.05). In Fig. 5, T2-weighted images showed the donor optic nerve had comparable morphology with the uninjured intraorbital optic nerve at 3 weeks after WET, whereas in the prechiasmatic optic nerves, DTI quantitation of the right injured optic nerve showed significantly lower FA and $\lambda_{//}$ by 54±6.1% and 24.9±5.7% respectively, and a significant increase in $\lambda_{\perp}$ by 83±29.5% compared to the left uninjured optic nerve (two-tailed paired t-tests, p<0.05). There was also an apparently larger $\lambda_{\perp}$ increase at 10 weeks than 3 weeks after WET (data not shown).

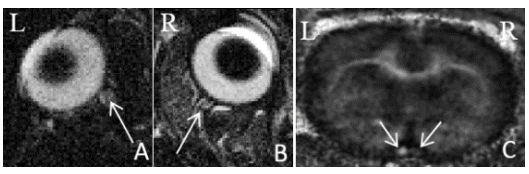

Fig. 5: (A, B) T2-weighted images of the uninjured intraorbital optic nerve (A) and donor optic nerve (B) (arrows) at 3 weeks after WET. Note the presence of cerebrospinal fluid in the optic nerve sheath surrounding the optic nerves; (C) Fractional anisotropy map of the prechiasmatic optic nerves from the recipient rat (arrows).

Discussion: Gd contrast agent has a low molecular weight and can mimic soluble aqueous humor components entering the anterior chamber via the leaky blood-aqueous barrier but not the blood-retinal or aqueous-vitreous barrier in normal eyes upon systemic administration [3-4]. Using Gd-enhanced MRI, we confirmed the presence of aqueous humor dynamics in the transplanted eye. In addition, the limited Gd enhancement in the vitreous of the transplanted eye indicated the preserved integrity of the blood-retinal and aqueous-vitreous barriers. Gd-enhanced ocular MRI also provided a non-invasive method to model the aqueous humor dynamics following WET. At 3 weeks after WET, Gd enhancement in the transplanted eye was generally weaker than the contralateral, uninjured eye, which was expected as the blood circulation of the eye might still be in the remodeling phase similar to the face transplant model [5]. At 10 weeks after WET, Gd peak enhancement appeared comparable between the left and right eyes, which revealed that aqueous humor dynamics was reestablished to its full potentials. Based on the FA, $\lambda_{//}$ and $\lambda_{\perp}$ differences between left and right prechiasmatic optic nerves, it is apparent that the prechiasmatic optic nerve of the recipient rats had compromised microstructural integrity after optic nerve transection and apposition.

Conclusions: A viable model of WET was established showing existing aqueous humor dynamics and preserved integrity of the blood-ocular and aqueous-vitreous barriers of the transplanted eye. Longitudinal Gd-MRI studies are ongoing to monitor the remodeling of aqueous humor dynamics in the current animals. Future DTI studies will examine approaches for regaining neuronal structure and function of the visual system to the current WET model.

References: [1] www.who.int/blindness, 2014; USA, 2012; [2] Washington K, Plast Reconstr Surg, 2009, 123:26S-33S; [3] Chan KC, Exp Eye Res, 2008, 87(4):334-41; [4] Ho LC, IOVS, 2014, 55(6):347-57; [5] Siemionow M, Plast Reconstr Surg, 2004, 113(5):1421-8