Diffusion Tensor Imaging detects and quantifies changes in permeability in the murine retina

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Introduction: Diabetic retinopathy is among the most common causes of blindness worldwide. The main causes of this are the breakdown of the blood-retinal barrier (BRB) and neovascularization or angiogenesis around the retina resulting from hypoxia and vascular endothelial growth factor (VEGF). Both these phenomena have been shown to be inter-related [1][2]. BRB breakdown can be characterized by increased barrier permeability. Our hypothesis is that diffusion tensor imaging (DTI) can be a valuable tool in quantifying changes in permeability in the BRB. By comparing the results of DTI in both control and pathogenic eyes, we can observe the direction and magnitude of water diffusion in the retina. Previously, we have demonstrated the feasibility of DTI in the murine retina. By comparing the diffusion of water between the controls and the pathologically altered eyes, we are able to quantify the changes in retinal diffusion following laser damage. As a model for pathogenic eyes we used eyes subjected to a focal (laser-induced) lesion, which induces similar conditions regarding the permeability in the retinal layers. Our experiments have been successful in discerning visible differences between the two samples thus validating the use of DTI techniques in detecting pathogenic changes in the retina.

Methods: C57 black (C57BL) mice were used for all the experiments. All animal procedures were performed in accordance with guidelines approved by the University of Florida Institutional Animal Care and Use Committee. A 750 MHz wide bore (89mm) NMR spectrometer (Bruker Instruments, Billerica, MA) was used for the study. The *in vivo* imaging setup included a portable small animal anesthesia machine, a respiratory monitoring system, and forced hot (35°C) air heating to maintain animal body temperature within physiologic limits. A linear square surface RF coil with dimensions 1 cm x 1 cm was used for *in vivo* imaging. A 21-direction high angular resolution diffusion imaging (HARDI 21) sequence was implemented using a gradient strength of 800 mT/m, resulting in a b-value of 970. The imaging parameters were TR=2500 ms, TE=14.3 ms, SW=67.5 kHz, matrix size=128 x 96, NA=1, slice thickness=0.25 mm. For the *in vitro* studies, the excised eyes were fixed in formalin solution (3% w/v) immediately following extraction. 24 hours prior to imaging, the eyes were transferred into cold phosphate buffered saline (PBS) solution to remove the fixative. On the day of the experiment, the eyes were transferred to a 5 mm NMR tube and suspended in Fluorinert™ (FC-43; 3M Corp.) to remove extraneous signals from the bathing medium. The control eyes were imaged using the afore mentioned HARDI 21 sequence as well as a 7-direction DT sequence with b-values ranging from 110 to 700 with similar imaging parameters except for higher averaging. The lasered eyes were imaged using a 30-direction DTI sequence with constant b-value of 800, TR=2500 ms, TE=12.7 ms, SW= 101 kHz, Δ=8 ms, δ=1.5 ms, matrix size=128 x 128, NA=4, slice thickness=0.20 mm. All image processing and analysis was done using FLTView software developed at the University of Florida.

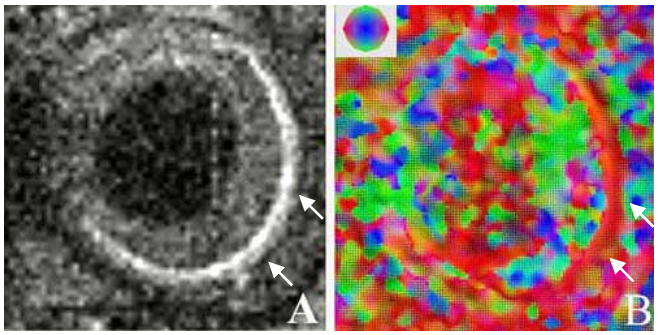


Fig 1: [A] DWI of in vivo eye with visible retina [B] Color map showing consistency in the direction of principal diffusion in the

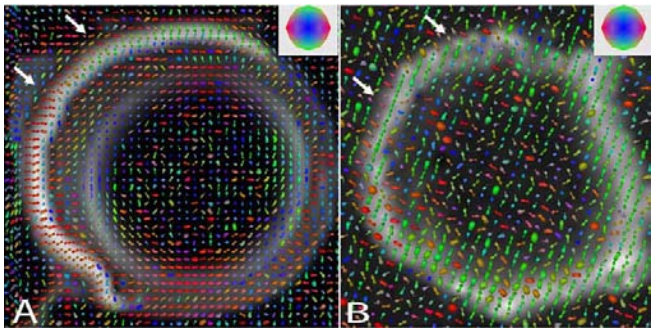
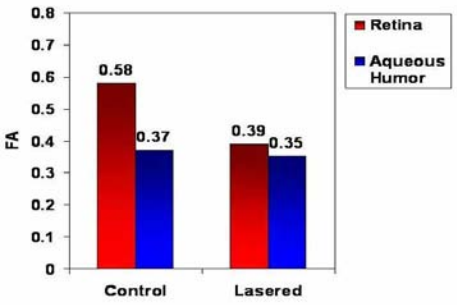


Fig 2: DT Maps [A] Control Eye and [B] Lasered Eye

Results and Discussion: Fig.1.A is a diffusion weighted *in vivo* scan. Though the noise level is understandably higher due to a short image acquisition period, the retina (marked by white arrows) is clearly distinguishable. The aqueous humor is present just beneath the retina. Fig 1.B is a color map of the same scan showing direction of principal diffusion in the retina. Though an overall non-uniformity of tensors exists due to varied anatomy, and lack of averaging, the tensors along the retina show a consistency in direction, that is, the diffusion is back and forth between the outer and inner layers. Fig. 2.A and 2.B are tensor maps of a control and lasered eye respectively. The control retina is clearly demarcated displaying distinctly higher fractional anisotropy (FA) than the aqueous humor. The direction of diffusion is much more defined in this case, yet consistent with the *in vivo* image. In the lasered eye (Fig.2.B), the boundaries of the retina are less distinct due to the breakdown of the blood retinal barrier. This is validated by a statistical decrease in FA in both the retina as well as the aqueous humor observed especially around the laser damage site. Fig.3 is a graphical representation of the difference in FA values between the retina and aqueous humor in the control and lasered eyes respectively. FA means and standard deviation values are listed in the table below. As is observed, the difference in FA is considerably higher in the control eye, but is much lesser in the lasered eye.



	Control		Lasered	
	Mean FA	Std dev	Mean FA	Std dev
Retina	0.57	0.12	0.39	0.12
Aq.Humor	0.37	0.11	0.35	0.07

Fig 3: Graph comparing FA values of the retina and aqueous humor of Control v/s Lasered eyes.

Conclusion: The fact that pathogenic retinas exhibit increased permeability due to the breakdown of the blood retinal barrier is established. DTI is shown to be capable of detecting and quantifying changes in permeability in the murine retina. Further studies involving imaging live animals with diabetic retinopathy is required to corroborate our results. Future research in this field will aid in detecting early onset of pathogenic conditions in the retina.

References: [1] David A. Antonetti, et al, Vol. 47, Dec 1998 [2] Eric Pierce, Robert Avery et al, Proc. Natl. Acad. Sci. USA, Vol. 92, pp. 905-909, January 1995, Biochemistry

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