Multi-layers structure of mouse retina detected using T2 and ADC in vivo

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Introduction:

Retina is the sensory membrane lining the eye. It functions as the immediate instrument of vision receiving the image through the lens and converting it into chemical and nervous signals reaching the brain. The diagnosis of retina related diseases depends on the ability of detecting morphological and functional changes of the structure. In addition, transgenic mouse models of retina diseases provide invaluable opportunities for better understanding of the underlying pathophysiology and developing novel therapeutic interventions. However, reports on non-invasive MRI characterization of the mouse retina have been scarce. The objective of this study is to evaluate the feasibility of non-invasive MRI methods, including T2 and ADC, for in-vivo characterization of retina in normal mice. These data would provide a baseline for future efforts to study retinal integrity in murine models of retinopathy and possibly in the clinic.

Method:

Animal Model: A total of six adult C57/BL6 mice were used. Mice were anesthetized with an initial i.p. dose of Ketamine(87mg/Kg)/Xylazine(13mg/Kg), followed by i.p. infusion of Ketamine(54mg/Kg/hr)/Xylazine(8.2mg/Kg/hr) 50 min after the initial injection. The body temperature of mice was kept at 37°C and the respiration was monitored using an MR compatible small animal heating and monitoring system with a feedback control (SA instruments, NY).

MRI: MRI of the mouse eye was performed using a Varian 11.7T UNITY-INOVA spectrometer employing a custom-built solenoid coil. Scout images were acquired to locate a transverse slice through optical nerve. Multiple T2-weighted images of mouse eye (n=3) were acquired using a standard spin-echo sequence with the following parameters: TR, 1500ms; TE, 21, 28, 38 and 50ms. Diffusion weighted spin-echo images of mouse eye (n=6) were acquired with diffusion weighting gradients applied in three directions, i.e., in-plane parallel to the optical nerve (||), in-plane perpendicular to the optical nerve (⊥) , and out of the image plane (⊙), using a spin-echo diffusion weighed sequence with the following diffusion specific parameters: TR,1200ms; TE,35ms; Δ, 20ms; δ, 7ms; b-value, 0 and 2007 s/mm^2. Other acquisition parameters common for T2 weighted and diffusion weighted imaging were: number of averages, 4; slice thickness, 400μm; FOV, 6 × 6mm^2; data matrix, 128 × 128 zero filled to 256 × 256, resulting in a 46 × 46 μm^2 in-plane resolution.

Histology: Mice were euthanized at the conclusion of MRI with eyes excised and fixed with formalin. 4μm thick slices of the mouse eye were sectioned through optical disk and perpendicular to retina for qualitative comparison with MRI results. These sections were stained with hematoxylin and eosin to delineate the multiple layered structure of the retina.

Data Analysis: The retina and choroid were automatically segmented based on their high signal intensity on diffusion weighted image. Subsequently, retina and choroid were individually segmented using a threshold based on their signal intensity on T_2-weighted images. Apparent diffusion coefficients (ADC||, ADC⊥, and ADC⊙) in retina and choroid were calculated from diffusion-weighted images. T_2 map were calculated from T_2-weighted images.

Statistics: Paired student t-test was performed for statistical analysis. Statistical significance was accepted at $p < 0.05$.

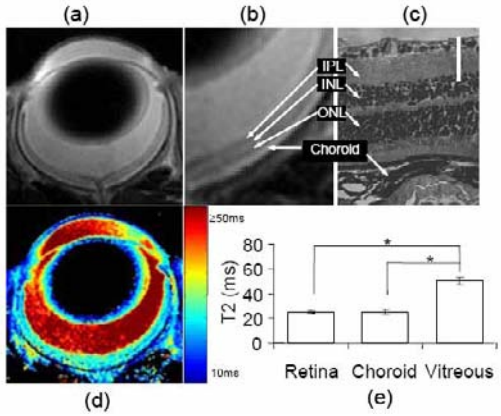

Figure 1 (a) T2 weighted image with 21ms echo time. (b) Magnified multiple layers of retina. (c) The histology diagram of retina, scale bar, 50μm. (d) T2 map of the mice eyeball (e) Relaxation times of Retina, Choroid and Vitreous, * $p < 0.05$ for paired comparison.

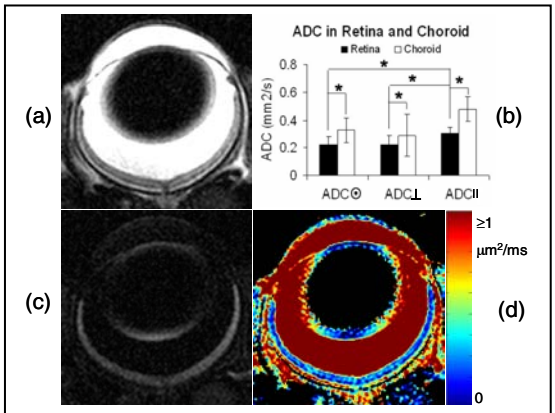

Figure 2 (a) B0 image (b) The quantification of ADCs in retina and choroid, * $p<0.05$ for paired comparison (c) Diffusion weighted image parallel to optical nerve (||) (d) ADC|| map.

Result:

Based on anatomical considerations, tentative retinal layer assignments are proposed, where the choroidal assignment in this work was based on the preliminary Gd-DTPA enhanced measurements (Fig 1). T2 in retina and choroid were not significantly different from each other. However, they were significantly lower than that in vitreous body (Fig 1). Retina and choroid were hyper-intense enhanced in diffusion weighted image (Fig 2c), indicating significantly restricted water diffusion in retina and choroid as compared to that in vitreous body. ADC in retina was significantly lower than that in choroid at all three directions. In retina, ADC|| was significantly higher than ADC⊥ and ADC⊙ (Fig 2b).

Discussion and Conclusion:

The current study presents the first in-vivo high-resolution measurement of T2 relaxation time and ADC in the mouse eye. The ratio of T2 between vitreous and retina and the ratio between ADC|| and ADC⊥ in retina were consistent with those in cat at 4.7T (1). The T2 value in individual tissue observed herein was consistent with previous observations in brain over a range of field strengths (2). Multiple layers were observed in retina on T2 weighted image while less clear in T2 map. This requires further investigation. ADC in retina is lower than that in choroid, which may be due to the high-density of cells in retina or due to faster choroid blood flow. Our findings suggest that high resolution T2 and ADC maps of the mouse retina are feasible. The observed retinal layers will provide an opportunity to investigate pathological process, such as retinal edema, of individual cell layers present in various retinal diseases.

Reference:

1. Shen et al, J Magn Reson Imaging 23: 465-472 (2006).
2. Graaf et al, Magnetic resonance in Medicine.56: 386-394(2006)