Manganese Alters Water Movement in Ocular Lens Detected by DTI in vivo

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Introduction The ocular lens, which has no blood vessels, relies on internally directed ion and water fluxes for its circulation, survival and transparency. The aquaporin (AQP) 0 is the major membrane channels of the outer fiber cells to account for the fluid circulation of lens (1), while the inner cells lose their organelles but retain high concentrations of crystallins and other proteins (2). The malfunction of AQP0 can cause cataract. A non-invasive method to detect the function of AQP0 is critical to improve the early diagnosis of cataract. In this study, we hypothesized that Diffusion Tensor Imaging (DTI) can detect AQP0 malfunction in ocular lens. Because AQP0 is Ca^{2+} dependent, in this study, we used Mn^{2+}, a Ca^{2+} analog, to alter the function of AQP0 in mouse ocular lens (3, 4). DTI was performed to evaluate the diffusion changes in the eyes affected by Mn^{2+}, compared to the controls.

Materials and Methods Seven 8-week-old female C57BL/6 mice were used. 1.0 M $MnCl_2$ solution was provided to the surface of the right eye on each mouse. After one hour, the remaining solution was carefully removed by lint-free tissue. At 24 hours after the $MnCl_2$ administration, T1-weighted imaging (T1WI) was taken using a Bruker 4.7T BioSpec animal scanner with TR of 250 ms, TE of 8 ms, FOV of 1.5cm, and data matrix of 128 x 128 (with zero-padding to 256 x 256), followed by DTI using spin echoes with TR 3 s, TE 29 ms, duration between a diffusion gradient pair 20 ms, diffusion gradient duration 3 ms, and six-direction diffusion scheme with b-values of 0 and 0.85 ms/μm^2. Using software written in Matlab (MathWorks, Natick, MA, USA), DTI indices including axial diffusivity (λ||), radial diffusivity (λ⊥), relative anisotropy (RA), and trace of the diffusion tensor (TR) were quantified.

Results Mn^{2+} caused enhanced T1-weighted signal (Mn-enhanced MRI, MEMRI) in lens, retina (**Fig. 1**), and optic nerves (**Fig. 2**). Via DTI, a decrease of λ⊥, an increase of RA, and a decrease of TR in the lens boundary (**Figs. 1 and 3**). No DTI change was found in retina and optic nerves, affected by Mn^{2+} (**Figs. 1-3**).

Discussion and Conclusion Due to the elongated shape of fiber cells and their layered arrangement (**Fig. 4**), lens fiber cell layers have known to produce high diffusion anisotropy detected by DTI, in which the radial diffusivity (λ⊥) is sensitive to water movements across cells. Our data showed a decrease of λ⊥ in the Mn^{2+}-affected lens boundary, suggesting that Mn^{2+} may alter the function of AQP0 causing a reduced water movement across the lens fiber cells. Because DTI did not change in Mn^{2+}-affected retina and optic nerves, the findings in lens boundary is not simply caused by increasing T1 in MRI signals. In conclusion, DTI was proved to be a potential marker to evaluate AQP functions *in vivo*.

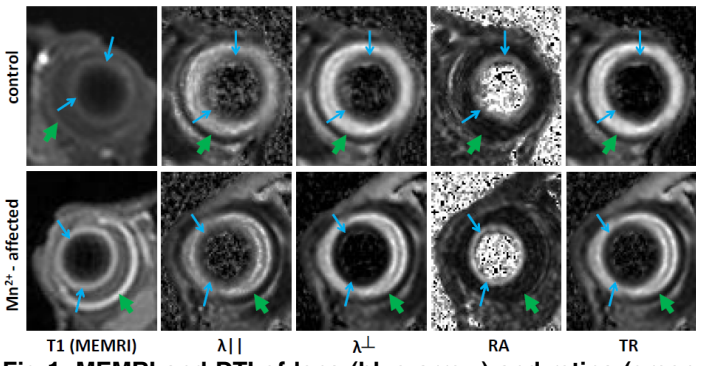

Fig 1. MEMRI and DTI of lens (blue arrow) and retina (green arrow) from Mn2+-affected and control eyes. Mn2+ caused a significant reduction of λ⊥, leading to increased RA and decreased TR in lens boundary.

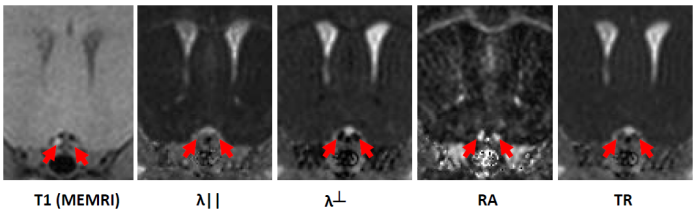

Fig 2. MEMRI and DTI of optic nerves (red arrow).

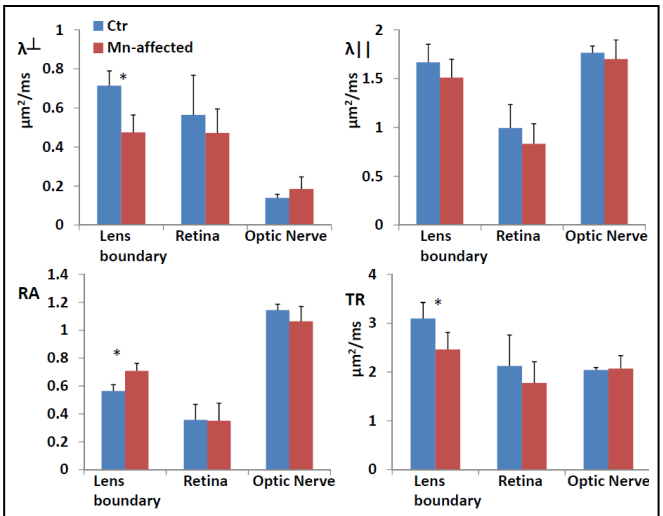

Fig 3. DTI of lens boundary, retina, and optic nerves affected by Mn^{2+}, compared to controls.

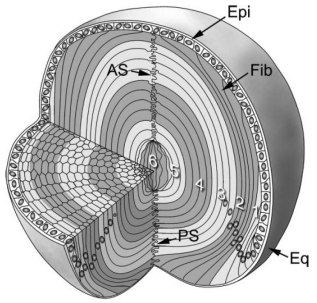

Fig 4. Mouse lens structure. Epi: epithelial cells, Fib: fiber cells, Eq: lens equator. AS: anterior sutures, and PS: posterior sutures. (*picture modified from Maria et al, the journal of biological chemistry 2009, 284 (20), pp. 13542–13550*)

References (1) McNulty et al, J. Physiol 2004, 559 883–98. (2) van Kamp et al, Exp. Eye Res. 1973, 17 417–26. (3) Sun et al, IOVS 2011, 52 3914–3920. (4) Sun et al, IOVS 2012, 53 4699-709

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