Negative MEMRI in Optic Nerve after Transient Retinal Ischemia

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Introduction Manganese (Mn) is currently the only MRI-sensitive marker for in vivo tract tracing in the central nervous system (CNS). Thus, Mn-enhanced MRI (MEMRI) has provided exciting findings of tracing neuronal circuits in living animals. Several studies have shown that Mn^{2+} enters cells through L-type voltage gated calcium channels and is transported along microtubules (1,2). Thus, it is possible impaired axonal transport as a consequence of axonal damage would manifest as a decrease in transport of Mn^{2+} detectable with *in vivo* MEMRI. To test this hypothesis, Eye-Drop MEMRI, noninvasive loading of Mn by eye drop for MEMRI, was conducted on mice after transient retinal ischemia. At 3 days after ischemia, significant axonal injury in optic nerve (ON) has been documented by histology and diffusion tensor imaging (DTI) (1, 2). In this study, both DTI and Eye-Drop MEMRI were employed to evaluate the damage of ON in mice at 3 days after retinal ischemia.

Materials and Methods Five C57BL/6 female mice, 6 – 8 weeks of age underwent transient retinal ischemia (3, 4) on the right eye of each mouse. The left eye was not cannulated. A cohort of five age-matched mice served as controls. At 3 days after ischemia, 10µl of 0.5 M $MnCl_2$ was loaded into the right eye by eye drop under anesthetization. After 1 hour, the remaining solution was carefully removed by a lint free tissue without touching the eye, and mice were returned to their cages. Twenty-four hours later, mice underwent T1-weighted imaging (T_1WI) with TR 0.4 s, TE 23 ms, NEX 8, slice thickness 0.5 mm, field-of-view 3 cm, and data matrix 256 × 256 (zero filled to 512 × 512). In addition to T_1WI, DTI was acquired with TR 1.5 s, TE 50 ms, Δ 25 ms, δ 10 ms, and NEX 4. Diffusion sensitizing gradients were applied along six directions with b-values of 0 and 0.85 ms/µm^2. The regions of interest (ROI), including left and right optic nerves (ON), optic tracts (OT), optic radiations (Rad), and visual cortex (Cort), were selected. As the mouse is a monocular species, the ROI were measured from the *right* ON, *left* OT, *left* Rad, and reaching the *left* Cort. The control pathway was measured in the same anatomical structures, but from the opposite hemisphere. The enhancement was quantified as (intensity-Mn2+ – intensity-control) / intensity-control.

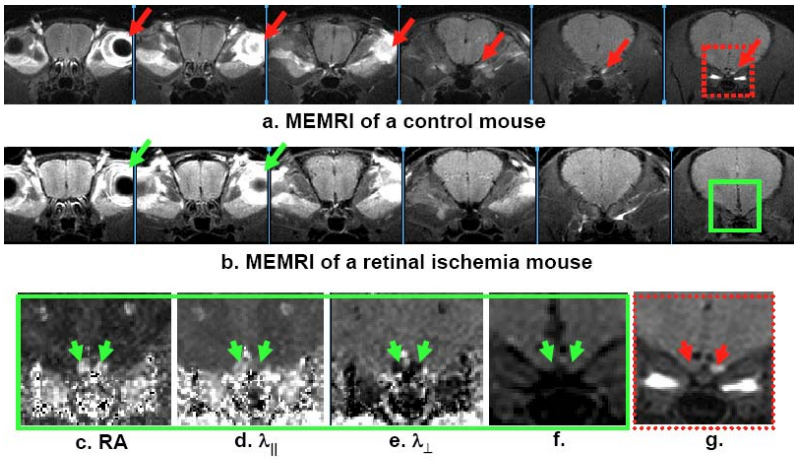

Fig. 1, MEMRI of a control (a, g) and retinal ischemia mice (b, f). The DTI of the retinal ischemia mouse (c, d, and e) showed decreased $\lambda_{||}$.

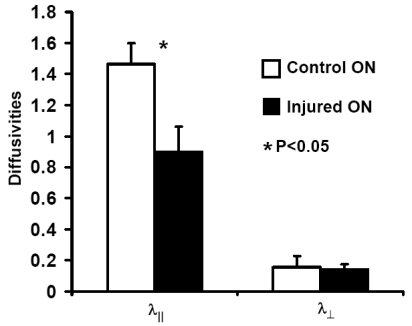

Fig. 2, $\lambda_{||}$ and $\lambda_{\perp}$ in ON at 3 days after retinal ischemia.

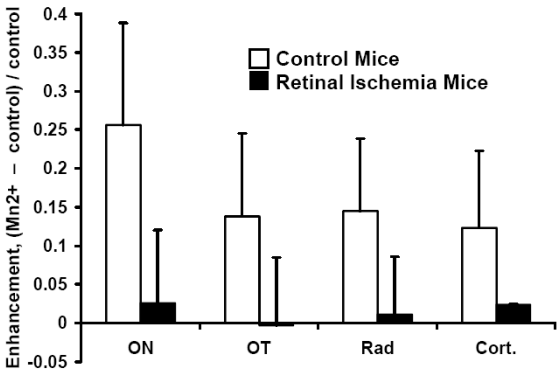

Fig. 3, MEMRI: No enhancement in the retinal ischemia mice.

Results Following Eye-Drop MEMRI, enhancement was clearly shown in visual pathway of control mice (Figs 1a & g). However, in retinal ischemia mice, the enhancement was clearly shown in vitreous space (green arrows in Fig. 1b), but the enhancement was not seen in the visual pathway as observed in the control mice (Figs. 1f, g and 3). DTI showed that retinal ischemia mice displayed a significant decrease (38%) in $\lambda_{||}$, suggestive of severe axonal damage in ON (Figs. 1d and 2).

Discussions and Conclusions

In this study, the damage of ON induced by retinal ischemia was evaluated with DTI and Eye-Drop MEMRI. The damage resulted in an absence of Eye-Drop MEMRI enhancement in the impaired visual system. Furthermore, a significant decrease in $\lambda_{||}$, but no change in $\lambda_{\perp}$ was seen with DTI, consistent with previously reported studies (3, 4). This study demonstrated that the uptake of Mn^{2+} may require functional neuronal cells (i.e., the retinal ganglion cells) and the intact axonal transport system. Although significant Mn^{2+} accumulated in the vitreous space (green arrows in Fig. 1b), no passive diffusion of the Mn2+ into the intra- and inter-cellular space of the visual pathway was observed following retinal ischemia. The loss of retinal ganglion cells or the impaired axonal transport system of the injured eye may be responsible for the lack of MEMRI enhancement. Further studies are necessary to determine the underlying mechanism of the observed MEMRI deficit, and its relationship to axonal damage detected with DTI.

References

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Acknowledgement: *NMSS: RG 3864, CA 1012-A-13; NIH: R01 NS 047592, R01 NS 054194.*