Evaluation of Glaucomatous Optic Nerve using in vivo Manganese-enhanced MRI

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INTRODUCTION: Glaucoma is a neurodegenerative disease of the optic nerve (ON) characterized by retinal ganglion cell (RGC) death, optic nerve head (ONH) damage, and progressive visual field loss [1]. While elevated intraocular pressure (IOP) is considered a major risk factor, the primary cause to the atrophic mechanisms is still unclear [2]. In this study, *in vivo* manganese-enhanced MRI (MEMRI) was applied to evaluate dynamically the Mn^{2+} enhancements along the visual pathway following an induction of ocular hypertension in rat models of chronic glaucoma. The goal is to examine the axonal transport in the glaucomatous optic nerve and to investigate into the optic nerve integrity in accordance with previous findings.

METHODS:

Experimental Procedures: Sprague-Dawley female rats (250-280g, N=11) were divided into 2 groups and were prepared to induce ocular hypertension unilaterally in the right eye by photocoagulation of episcleral and limbal veins using an argon laser [3]. A second laser treatment in the same setting was applied 7 days later to maintain a consistent IOP elevation by about 1.5 times above the normal level. Group A (n=6) and Group B (n=5) were scanned 2 weeks and 1 month, respectively, after first laser treatment. MEMRI was performed 2 to 5 hours (Group A), and 8 & 24 hours (Group B) after intravitreal injection of 50mM, 3μL $MnCl_2$ solution into both eyes. Throughout the experiments, the left ON served as a control.

3D MR Imaging: All MRI measurements were acquired utilizing a 7T Bruker scanner. Under inhaled isoflurane anaesthesia (2% induction and 1% maintenance), animals were kept warm on a heating pad at ~35°C and were imaged using an isotropic T1-weighted 3D RARE sequence with TR/TE = 300/6.6ms, flip angle =180°, RARE factor = 4. FOV = 3.24x3.24x2.47cm^3 and voxel resolution = 193x193x193μm^3. 2 averages were used and the total acquisition time was 39.5 minutes.

Data Analysis:

1. Measurement of ON profile near ONH: Rectangular regions of interest (ROIs) were drawn manually 0-3mm distal to the ONH at 0.2mm intervals along the ON, and the mean and standard deviation of the ROIs were found. The ROIs were copied for each sample. In order to compensate for B_1 inhomogeneity, each value was normalized to the unaffected muscle adjacent to the ON of the same slide. Signal-to-noise ratio (SNR) was calculated from $655xSI/SD_{air}$, where SI is the normalized signal intensity of the ROI and SD represents standard deviation [4].

2. Measurement of axonal transport rate index: Maximum intensity projection (MIP) was performed to a segmented volume embracing the entire ON segment from the ONH to the optic foramen in both eyes (Figure 1). 6 ROIs were drawn at 1mm intervals distal to the ONH. Axonal transport rate indices were calculated from the ratio of signal intensity of an ROI to that of the same ROI at the first scanning time point, and the values obtained between the normal and glaucomatous ONs were compared. All 3D images were reconstructed and co-registered before analysis.

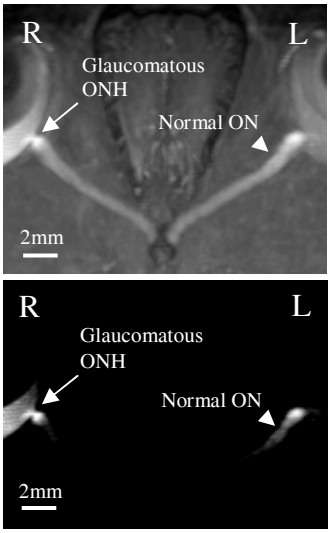

Figure 1: Typical MIP images of the optic nerves before (left) and after (right) contrast enhancement. A clear constriction was observed at the glaucomatous optic nerve head (ONH, arrow) compared to the normal one, while a brighter signal was found in the normal intraorbital optic nerve (ON, arrowhead). Image was acquired 4.5 hours after Mn^{2+} injection.

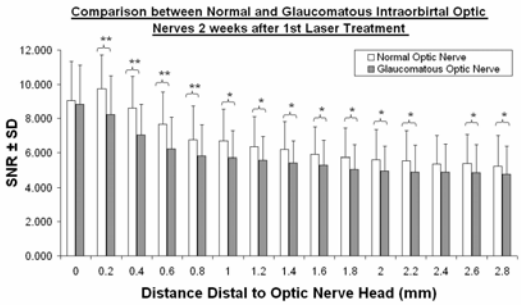

Figure 2: SNR vs distance from optic nerve head in glaucoma rats 2 weeks after laser treatment. Data were obtained 5 hours after Mn^{2+} injection. (Group A, n=6; paired t-test *p <.05, **p < .01).

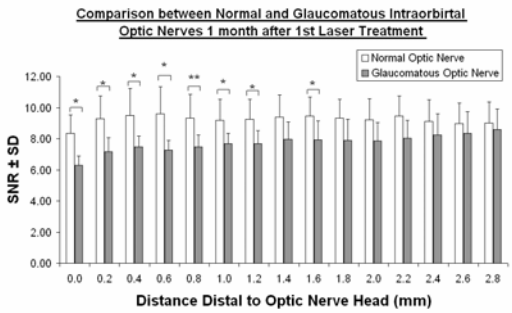

Figure 3: SNR vs distance from optic nerve head in glaucoma rats 1 month after laser treatment. Data were obtained 8 hours after Mn^{2+} injection. (Group B, n=5; paired t-test *p <.05, **p < .01)

RESULTS AND DISCUSSION:

1. ON profile near ONH: At 5 hours after Mn^{2+} injection, statistical evaluation found that the SNR near the ONH of the glaucomatous ON in Group A was lower than that of the normal ON by about 12% (Figures 1 & 2), while in Group B it was about 20% lower at 8 hours after Mn^{2+} injection (Figure 3). Given that loss of axons in the ON was observed in previous studies by approximately 19% 3 weeks after first laser treatment [5], the similar degree of SNR decrease had likely implicated an axonal density reduction in the glaucomatous ONs of our models.

2. Axonal transport rate indices: From 2 to 5 hours after Mn^{2+} injection, the signal intensities of different sections of the glaucomatous ONs in Group A were observed to increase at a lower rate than the corresponding normal ones. Significant differences between the normal and glaucomatous axonal transport rates were found in 3 out of 6 of the ROIs while more were found at later scanning time points. (data not shown). Mn^{2+} was known to travel along the microtubules by fast axonal transport [6]. The drop in the rate of Mn^{2+} transport might have suggested an interruption of axoplasmic flow along the glaucomatous ON, which would in turn interfere with the material supply necessary to maintain the distal axons and synapses in orthograde transport [7], causing axon death.

3. Morphological changes: Upon Mn^{2+} injection, out of the 6 glaucomatous rats observed in Group A, 5 showed a severe constriction at the glaucomatous ONH when comparing with the normal one (Figure 1). This appeared to indicate axonal loss in the superior and temporal regions of the ONH, as well as axonal swelling and accumulation of organelles, including Mn^{2+}-carrying microtubules, at the pores of the ONH [7,8]. On the other hand, the SNRs and the overall structures of the lateral geniculate nuclei and the superior colliculi in Group B were examined with insignificant differences (p for SNR = 0.27 and 0.38 respectively at 24 hours after Mn^{2+} injection). Further investigations will be performed on the long-term neurodegeneration in intracranial ON, lateral geniculate nucleus and visual cortex in this disease [9].

CONCLUSION: MEMRI can clearly detect abnormalities in the glaucomatous ON non-invasively especially near the ONH. This may help understand the disease mechanisms, monitor the effect of drug interventions to glaucoma models and complement the electrophysiological diagnoses of ON.

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