Axonal transport rate decreased at the onset of optic neuritis in EAE mice

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of central nervous system (CNS). Frequently, optic neuritis (ON) is an early symptom of MS[1]. Axonal transport deficits are an early event of many neurodegenerative diseases[2]. However, few reports have assessed axonal transport rate in MS or in experimental autoimmune encephalomyelitis (EAE). EAE induced in C57BL/6 mice is a widely used animal model of MS and exhibits many MS-like pathologies, including ON. Mn^{2+}, a Ca^{2+} analog, is paramagnetic to shorten T1 relaxation time and crosses cell membrane trough voltage-dependent Ca^{2+} channels. Hence, *in vivo* manganese-enhanced MRI (MEMRI) provides a way to investigate real-time axonal transport in rodent visual pathway. In this study, we measured visual acuity (VA) by optokinetic response (OKR) to define onset of ON in EAE mice[3]. Then, $MnCl_2$ was injected in the vitreous of sham and EAE mouse eyes, followed by series of time-lapse T1-weighted (T1W) images to compare axonal transport rate. The results suggested that axonal transport impairment was present and correlated with impaired visual functional and pathologies. The results of this study showed that axonal transport deficit is an early event of functional deficit of optic nerve in EAE mice. *In vivo* MEMRI is ideal to investigate axonal transport.

Materials and Methods

Animal Model: EAE was induced in 10 female eight-week-old C57BL/6 mice with MOG_{35-55} peptide in incomplete Freund's adjuvant emulsion. The other six age-matched control mice (sham group) underwent the same procedure without MOG_{35-55} immunization. Daily visual acuity (VA) was measured and MEMRI was performed at VA ≤ 0.25 cycle/degree (c/d). According to severity of ON, mice were separated into three groups, sham (VA = 0.35/0.4 c/d), moderate (VA = 0.2/0.25 c/d), and severe (VA = 0 c/d) ON. ***Intravitreal injection:*** A dose of 50 nmol $MnCl_2$ (0.25μL of 0.2 M) was delivered to vitreous space at a rate of 3μL/min with 34-gaugue needle[4]. ***MEMRI:*** A pair of 8-cm diameter volume and 1.7-cm diameter surface active-decoupled coils was used. Experiments were performed on a 4.7-T Agilent small-animal MR scanner with a standard 3D gradient echo sequence with the following parameters: TR = 15 ms, TE = 2.63 ms, flip angle = 20°, FOV = 15 × 15 × 22 mm^3, matrix size = 128 × 128 × 64 (zero-filled to 256 × 256 × 128), acquisition time = 32.8 minutes, number of signal average = 16, and ten successive sets of 3D-T1W images were captured at ~0.55 – 5.5 hours post-injection. ***B1-inhomogeneity correction:*** 3D-T1W image of a 2% agar gel phantom was placed underneath surface coil using the same acquisition MEMRI parameters with 64 averages. The raw 3D-T1W image of mouse brain was divided by the 3D-T1W image of phantom voxel by voxel using ImageJ to correct B1-inhomogeneity. ***Data analysis:*** The corrected 3D-T1W image set was adjusted and rotated to oblique plane that covered retina and optic nerves before chiasm (Fig. 1). **Accumulation rate calculation:** ROIs (region of interest) were drawn on Mn^{2+}-loading optic nerves and reference area for all oblique corrected T1W images (Fig. 2A). The slope of normalized intensity, calculated by ratio of Mn^{2+}-loading ROI and reference area, was accumulation rate (Fig. 2C and 2E). **Transport rate calculation:** ROI lines were drawn on Mn^{2+}-loading and contralateral optic nerves for all oblique corrected T1W images (Fig. 2B). Arrival of Mn^{2+} was determined by intensity of voxel ≥ threshold (mean + 2SD of contralateral ROI line). The slope of normalized displacement (number of voxels with Mn^{2+} arrival), normalized by the whole optic-nerve ROI line, was transport rate. After converting with image resolution, transport rate was converted to millimeter per hour (Fig. 2 D and F). ***Histology:*** mice were perfusion fixed immediately after the *in vivo* MEMRI for immunohistochemical staining of the optic nerve.

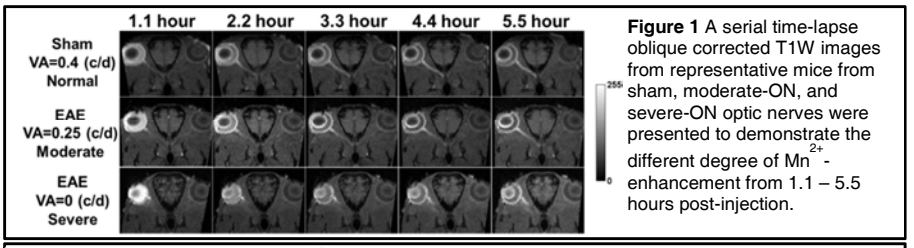

Figure 1 A serial time-lapse oblique corrected T1W images from representative mice from sham, moderate-ON, and severe-ON optic nerves were presented to demonstrate the different degree of Mn^{2+}-enhancement from 1.1 – 5.5 hours post-injection.

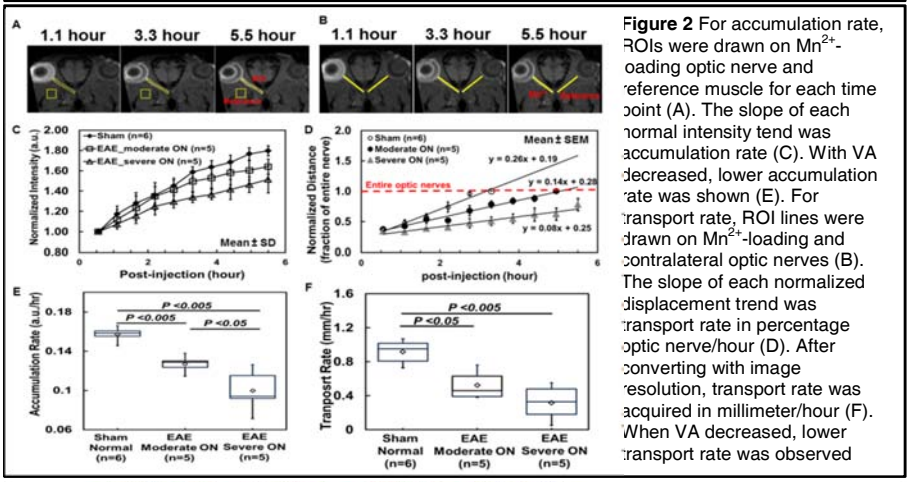

Figure 2 For accumulation rate, ROIs were drawn on Mn^{2+}-loading optic nerve and reference muscle for each time point (A). The slope of each normal intensity tend was accumulation rate (C). With VA decreased, lower accumulation rate was shown (E). For transport rate, ROI lines were drawn on Mn^{2+}-loading and contralateral optic nerves (B). The slope of each normalized displacement trend was transport rate in percentage optic nerve/hour (D). After converting with image resolution, transport rate was acquired in millimeter/hour (F). When VA decreased, lower transport rate was observed

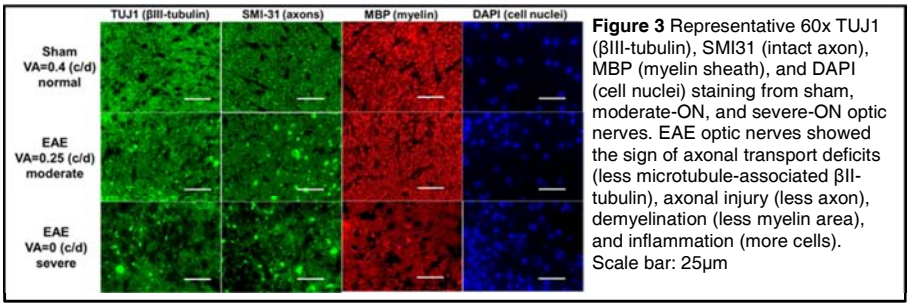

Figure 3 Representative 60x TUJ1 (βIII-tubulin), SMI31 (intact axon), MBP (myelin sheath), and DAPI (cell nuclei) staining from sham, moderate-ON, and severe-ON optic nerves. EAE optic nerves showed the sign of axonal transport deficits (less microtubule-associated βII-tubulin), axonal injury (less axon), demyelination (less myelin area), and inflammation (more cells). Scale bar: 25μm

Results

Compared to sham optic nerves, Mn^{2+} accumulation rate was significantly decreased by 19% ($p < 0.005$, moderate EAe) and 38% ($p < 0.005$, severe EAE) (Fig. 2E). The accumulation rate was 23% ($p < 0.05$) slower in the EAE mice with severe ON than that with the moderate ON (Fig. 2E). Compared to sham optic nerves, Mn^{2+} transport rate was significant decreased by 43% ($p < 0.05$, moderate EAE) and 65% ($p < 0.005$, severe EAE). Representative TUJ1(microtubule-associated βIII-tubulin, staining the base structure for kinesin motor protein movement)[5], SMI31 (intact axon), MBP (myelin sheath), and DAPI (cell nuclei) stains showed that EAE optic nerves developed axonal transport deficits, axonal injury, demyelination, and inflammation.

Conclusion

Our results demonstrate that MEMRI can provide an *in vivo* real-time quantification of axonal transport in EAE mice at ON onset. In addition to morphological changes, functional deficits, such as axonal transport disruption, also occurred at this stage. Preventing the worsening or curing the early axonal transport deficit may be a potential treatment target to halt the degeneration of axons in MS. Our results showed the first quantitative assessment of axonal transport in EAE mice suggesting that MRMRI is suitable for pre-clinical drug tests.

Reference

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