Optic neuritis pathology detected with directional water diffusivities by MRI in a murine EAE model

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Introduction

Optic neuritis is the commonest relapse manifestation of human multiple sclerosis. It has been recognized that optic neurits also occurs in the myelin oligodendrocytic glycoprotein (MOG) -induced murine experimental autoimmune encephalomyelitis (EAE) model. It has been proposed that directional water diffusivities along and across the white matter fibre can be used as surrogate markers of different white matter pathologies [1]. Thus, the aim of the present study was to assess the feasibility of examining optic neuritis pathology in the MOG-EAE mouse with directional diffusion weighted MRI and examine their histological correlates.

Methods

Eight C57Bl6 mice with MOG35-55 EAE at 8 to 10 weeks of age underwent MRI scan at EAE day 20. Five healthy age-matched mice were scanned as control. After MRI scan, animals were sacrificed immediately and the optic nerves were dissected for histological processing and analysis. In-vivo MRI scanning was performed on a 4.7T Bruker scanner. To image the optic nerves in longitudinal orientation, we used the bilateral junctions of the optic nerves with each globe and optic chiasm as anatomic landmarks to determine the oblique slice containing the optic nerves from the orbits to the optic chiasm. Instead of full-DTI, a spin echo DWI sequence with two orthogonal diffusion encoding gradients was employed, which were set parallel and perpendicular to one targeted nerve respectively. Sequence parameters: TR/TE = 1000/30 ms, 8 NEX, slice thickness = 0.5 mm, FOV = 2 × 2 cm^2, matrix size 256 × 128 and b values of 0 and 700 s/mm^2. Raw images were processed and ADC maps were generated using the commercial software MIStar. In order to avoid the contamination effect induced by the signal from orbital fat, the pre-chiasmal segment of optic nerve were selected as region of interest (ROI) and were initially outlined by manual tracing on DW images and further edited on generated maps. Group differences were examined using Student's t-test.

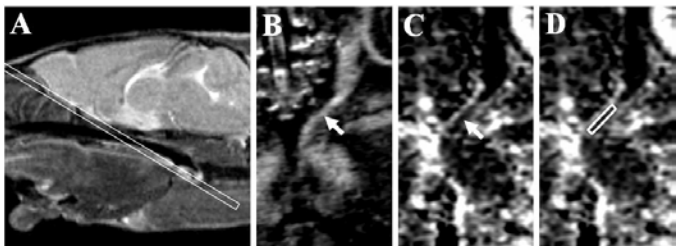

Fig. 1 (A) Location of the oblique slice containing optic nerve. DW image (B) and $ADC_{\perp}$ map (C) demonstrate good contrast of the nerve against neighboring tissue for ROI identification (white arrow). (D) Indication of the ROI in data analysis.

Results and Discussion

Raw DWIs and $ADC_{\perp}$ map demonstrate that the contrast of optic nerve against neighbouring tissues allows accurate identification of this structure in the longitudinal plane (Fig. 1B,C). In comparison with healthy controls, average water diffusivity parallel to the nerves ($ADC_{\parallel}$) of the optic neuritis nerves was significantly decreased by 25%, while water diffusivity perpendicular to the fibre ($ADC_{\perp}$) did not show significant change (Fig. 2). Consistent with previous reports that reduction of axial diffusivity in white matter tracts is associated with axonal injury, our histological analysis revealed the presence of axons stained with antibody against hypophosphorylated neurofilament (green fluorescence) and axonal size atrophy in methylene blue stained cross-sections in optic neuritis. Also extensive cellular infiltration was detected histologically (Fig. 3). These pathologies may all contribute to the observed $ADC_{\parallel}$ reduction. However, the underlying mechanisms account for $ADC_{\perp}$ changes are more complex. In the current study, the barriers for water diffusion across the fibre were relatively intact because no demyelination was observed and axonal alignment become tighter due to axonal shrinkage [2], further, the neurofilaments damage in optic neuritis probably induced an increase of intra-axonal viscosity.

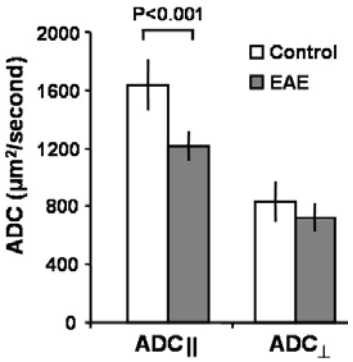

Fig. 2 Parallel ADC in nerves with optic neuritis (n=8) significantly decreased at EAE day 20 in comparison with healthy mice (n=5). Perpendicular $ADC_{\perp}$ did not show significant change.

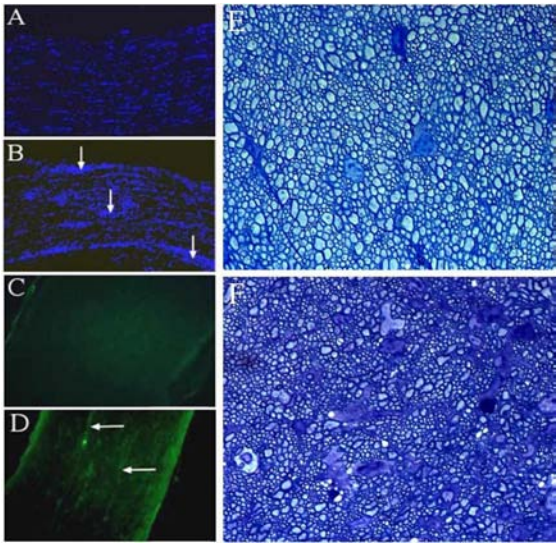

Fig. 3 DAPI-stained longitudinal sections reveal extensive inflammatory infiltration in optic neuritis (B) compared with healthy nerve (A). Axonal injury was detected with Ab against hypophosphorylated neurofilaments in optic neuritis (D) but not in healthy nerve (C). Methylene-blue stained cross-sections show marked increased cellularity and shrinkage of axon size but without demyelination.

Conclusion

Our results indicate marked reduction of axial water diffusivity in murine optic neuritis. Detailed histological examination shows that $ADC_{\parallel}$ is a surrogate marker in assessing axonal injury. $ADC_{\perp}$ is not a marker for axonal injury in the absence of demyelination.

References

[1] Song et al. *NeuroImage.* 2003; 20: 1714-22 [2] Bealieu et al. *NMR Biomed.* 2002; 15: 435-55