

Treatment Efficacy of FTY720 on Experimental Autoimmune Encephalomyelitis Mice Assessed by in Vivo Diffusion Basis Spectrum Imaging

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease with axonal injury causing permanent neurological disabilities. Diffusion MRI has been widely employed to non-invasively assess the severity of CNS axon injury and demyelination. By resolving diffusion weighted water signals resulting from white-matter structure (crossing fibers), CSF partial volume, and MS-associated pathologies (axonal injury, demyelination, and inflammation) as multiple-tensor components, diffusion basis spectrum imaging (DBSI) provides quantitative measures of inflammation associated cellularity and edematous water removing the confounding artifact of inflammation on the measured directional diffusivity.¹ Taking advantage of the simple white matter structure of spinal cord and optic nerves, the diffusion weighting scheme employed for DBSI may be reduced while still preserving the computation accuracy. In this work, DBSI with reduced diffusion scheme was applied in vivo, to evaluate the treatment effect of FTY720 on EAE-affected mouse optic nerves, by non-invasively assessing the extent of inflammation as well as axon and myelin integrity.

Methods

Animal preparation: To induce EAE, 10 female C57BL/6 mice were injected with 50µg MOG₃₅₋₅₅ peptide emulsified in incomplete Freund's adjuvant containing 2.5 mg/ml mycobacterium tuberculosis. Mice were also injected with 200 ng pertussis toxin in PBS on the day of immunization and again two days later. Five mice received 1 mg/kg FTY720 orally on a daily basis from the day of immunization until the study end point. Another five mice received saline. Five additional mice served as age and sex matched naïve controls. All mice were evaluated for visual acuity (VA) and clinical score (CS) daily. **MRI:** All mice underwent in vivo DBSI examination on a 4.7T scanner. A multi-echo spin-echo diffusion weighted sequence was employed with actively decoupled volume (6-cm inner diameter, RF excitation) and surface coil (1.5-cm diameter, signal receive). The overall set up is similar to that described previously.² All procedures performed were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee. A diffusion weighted mid-sagittal image was acquired to visualize mouse optic nerves. Then, one oblique image plan perpendicular to the optic nerve was acquired using a multi-echo spin-echo

diffusion-weighted sequence with the following parameters (Fig. 1): TR, 1.5 s, TE, 50 ms, Δ , 18 ms, and δ , 6 ms. The slice thickness was 0.8 mm covering the middle-third of the optical nerve, field of view was $2.25 \times 2.25 \text{ cm}^2$, and matrix size was 192×192 (zero-filled to 384×384). Diffusion gradients were applied along twenty-nine directions with distinct b -values, $b\text{-max} = 2.2 \text{ ms}/\mu\text{m}^2$.

Results

All five mice in the saline-treated EAE group developed optic neuritis indicated by decreased VA (≤ 0.25), while no mice from the group treated preventatively with FTY720 developed optic neuritis by the same criteria (data not shown). Significantly decreased $\lambda_{||}$ and increased λ_{\perp} of the optic nerves from a representative saline-treated EAE mouse (Fig. 2B) were consistent with previously reported findings in EAE.² Representative in vivo $\lambda_{||}$ and λ_{\perp} maps of optic nerves from FTY720-treated EAE mice demonstrated that FTY720 preserved white matter integrity (Fig. 2C). In addition, the cellularity and edema water maps also reflect the known anti-inflammatory action of FTY720. The end-stage values of $\lambda_{||}$, λ_{\perp} , cell ratio and edema water ratio of optic nerves from saline-treated EAE mice were 83% (indicating axonal injury), 151% (indicating demyelination), 111% (indicating cell infiltration) and 385% (indicating edema) of the control values, respectively. For the FTY720 treated group, $\lambda_{||}$, λ_{\perp} , cell ratio and edema water ratio values were 92%, 102%, 89% and 187% of the control values, respectively (Fig. 3).

Conclusion

Prophylactic treatment with FTY720 prevented optic neuritis in mice induced to develop EAE. FTY720 preserved axon and myelin integrity while at the same time suppressed inflammation. The optimized diffusion scheme allowed scan time shortening, by two-thirds comparing with the initial report.¹ This preliminary study suggested that white matter integrity markers ($\lambda_{||}$ and λ_{\perp}) and inflammation associated markers (cell and edema water ratio) derived from this in vivo 29-direction DBSI may be used to evaluate the efficacy of disease modifying interventions in EAE and potentially MS.

References

1. Wang Y et al, Brain, 134: 3590-3601 (2011). 2. Sun et al., Neurobiol Dis., 28: 30-8 (2007).

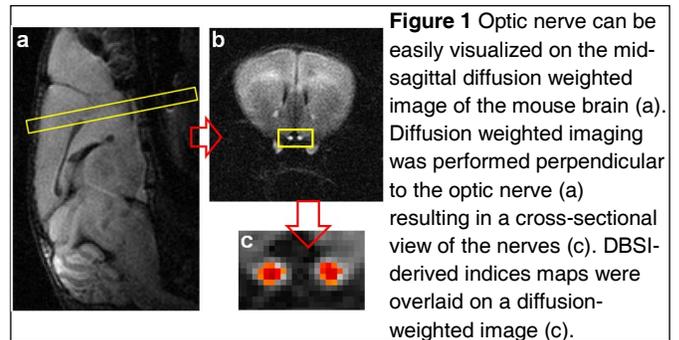


Figure 1 Optic nerve can be easily visualized on the mid-sagittal diffusion weighted image of the mouse brain (a). Diffusion weighted imaging was performed perpendicular to the optic nerve (a) resulting in a cross-sectional view of the nerves (c). DBSI-derived indices maps were overlaid on a diffusion-weighted image (c).

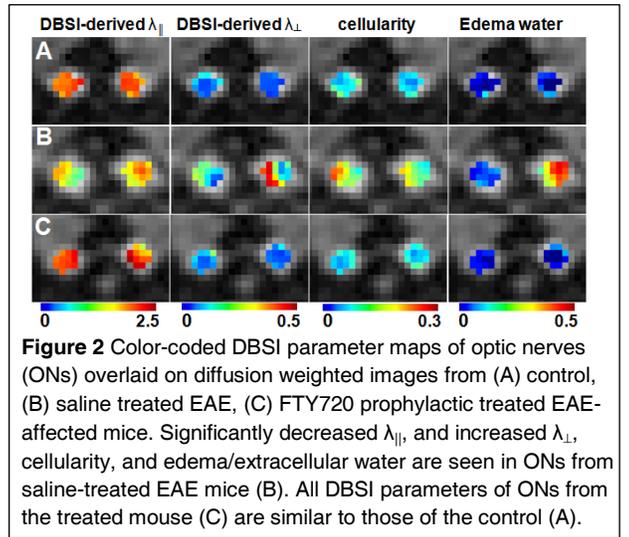


Figure 2 Color-coded DBSI parameter maps of optic nerves (ONs) overlaid on diffusion weighted images from (A) control, (B) saline treated EAE, (C) FTY720 prophylactic treated EAE-affected mice. Significantly decreased $\lambda_{||}$, and increased λ_{\perp} , cellularity, and edema/extracellular water are seen in ONs from saline-treated EAE mice (B). All DBSI parameters of ONs from the treated mouse (C) are similar to those of the control (A).

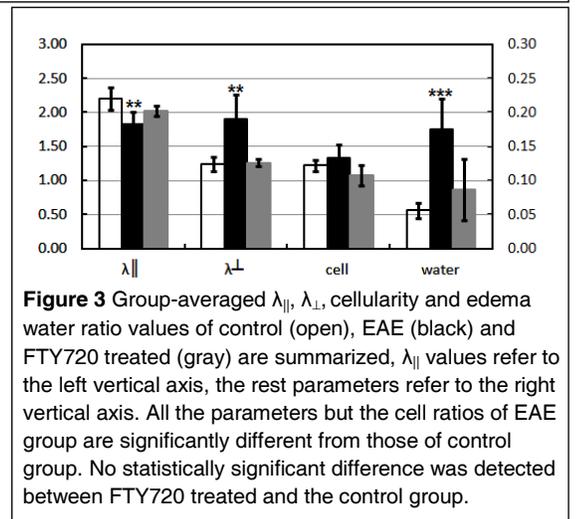


Figure 3 Group-averaged $\lambda_{||}$, λ_{\perp} , cellularity and edema water ratio values of control (open), EAE (black) and FTY720 treated (gray) are summarized, $\lambda_{||}$ values refer to the left vertical axis, the rest parameters refer to the right vertical axis. All the parameters but the cell ratios of EAE group are significantly different from those of control group. No statistically significant difference was detected between FTY720 treated and the control group.