

Assessing Anti-inflammation and Axonal Preservation Effect of FTY720 Using Diffusion MRI

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Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease with axonal injury causing permanent neurological disabilities. Based on accumulating literature evidence, the MS disability associated with acute MS relapses is likely related to the combined effect of the underlying inflammation, axonal injury, and demyelination, while long-term MS disability is likely due to the extent of permanent axonal damage, independent of the frequency or severity of relapses. Previously, diffusion tensor imaging (DTI) detected λ_{\parallel} decrease has been associated with axonal injury and dysfunction, and increased λ_{\perp} was associated with myelin injury in mouse models of white matter injury. Unfortunately, the DTI model does not address the confounding effects of inflammation-associated vasogenic edema (resulting in overestimated apparent diffusion coefficient (ADC), and underestimated white-matter tract diffusion anisotropy) or inflammatory cell infiltration (leading to a decreased overall ADC and underestimated diffusion anisotropy in the white matter). The diffusion properties of anisotropic structures derived using DTI lose specificity and sensitivity in the presence of increasing pathological and anatomical complexity. Herein, both DTI and diffusion basis spectrum imaging¹ (DBSI, a novel approach using a data-driven model-selection procedure to resolve multiple-tensor water diffusion resulting from both complex white-matter structure and MS-associated pathologies) analyses were employed to assess the anti-inflammation and axonal preservation efficacy of FTY720 in experimental autoimmune encephalomyelitis (EAE) mice.

Methods

Animal preparation: EAE was induced in C57BL/6 mice via injection of myelin oligodendrocyte glycoprotein (MOG₃₅₋₅₅) peptide emulsified in incomplete Freund's adjuvant with *Mycobacterium tuberculosis*. Control mice received incomplete Freund's and *Mycobacterium tuberculosis* in the absence of MOG. Daily treatment immediately after MOG immunization with vehicle and 3 mg/kg FTY720 was carried out on EAE mice. Control mice received no treatments (N = 5). **MRI:** At the study end point (28 days after FTY720 treatment), mice were subjected to intra-cardiac perfusion fixation using 0.01M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1M PBS. Mouse vertebral columns were excised, post-fixed overnight. Fixed mice cords underwent ex vivo DBSI examination on a 4.7 T scanner. A solenoid coil was used as both transmit and receive coil. Images of 4 contiguous transverse slices covering T11 through L1 vertebrae were acquired using the following parameters: TR 1.0 sec, TE 38 ms, Δ 20 ms, δ 5 ms, slice thickness 2.0 mm, zero filled spatial resolution (38 μ m x 38 μ m), total data acquisition time ~ 3.0 hr, diffusion gradient were applied along 99 directions on a 3D grid with maximum b value of 3000 s/mm².

Results and Discussions

Representative ex vivo DBSI derived maps of cellularity (restricted isotropic diffusion component) and vasogenic edema (non-restricted isotropic diffusion component) fractions, and DTI-derived relative anisotropy (RA) maps, at T13 vertebral segment of spinal cord from control and EAE with and without FTY720 treatment, qualitatively demonstrate the association of decreased RA with increased cell and edema fractions (Fig. 1). The axonal preservation of FTY720 treatment was apparent, evidenced by both DTI and DBSI derived axial and radial diffusivity (Fig. 2A). Without FTY720 treatment, EAE mice exhibited significantly decreased axial diffusivity and increased radial diffusivity reflecting extensive axon and myelin injury. The presence of inflammation was reflected by DBSI determined increase in cellularity and vasogenic edema fractions (Fig. 2B). Results demonstrated that FTY720 prophylactic treatment effectively reduce inflammation in EAE mice (Fig. 2B), in addition to preserving axonal integrity (Fig. 2A). Though both DBSI and DTI-derived diffusivities reflected axon and myelin damage in EAE, DBSI derived λ_{\parallel} was 40-60% higher and λ_{\perp} was 20-30% lower than those derived by DTI. The observed discrepancies probably result from the presence of inflammation associated cellularity increase and vasogenic edema in EAE, as well as the resident cells and inter-axonal space in the control mice. Our previous studies suggested that cellularity has negligible effect on radial diffusivity while edema has insignificant effect of axial diffusivity.¹ Thus, axial diffusivity derived by both DTI and DBSI was correlated with increased cellularity; radial diffusivity with the edema fraction of the spinal cord (Fig. 3).

Although both DTI and DBSI derived measurements correlate with inflammation suggesting the co-existence of inflammation and axon and myelin damages, axial diffusivity derived using DTI correlated with cellularity ($r = 0.90$) stronger than that by DBSI ($r = 0.80$). Similarly, DTI derived radial diffusivity correlated with edema ($r = 0.93$) than that by DBSI ($r = 0.81$). Results suggest that inflammation indeed impact DTI findings.

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References 1. Wang et al, Brain, in press (2011).

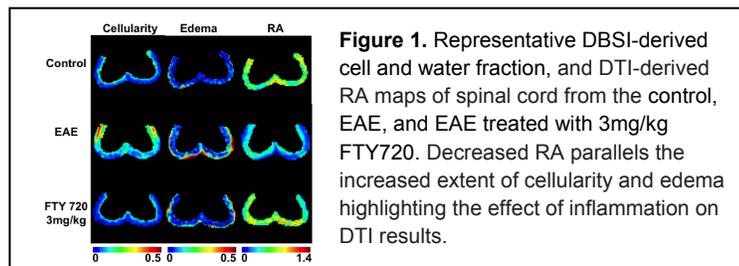


Figure 1. Representative DBSI-derived cell and water fraction, and DTI-derived RA maps of spinal cord from the control, EAE, and EAE treated with 3mg/kg FTY720. Decreased RA parallels the increased extent of cellularity and edema highlighting the effect of inflammation on DTI results.

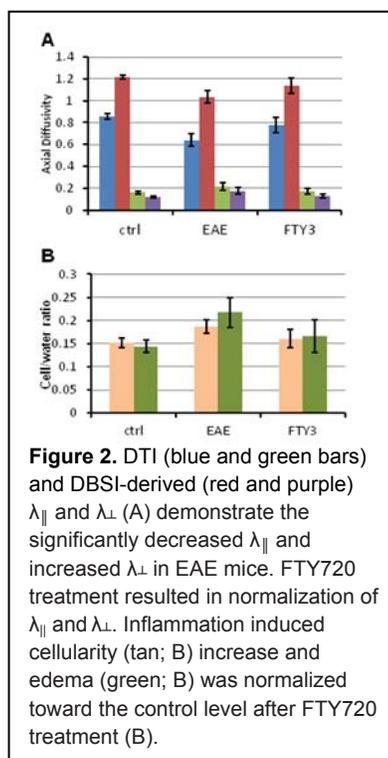


Figure 2. DTI (blue and green bars) and DBSI-derived (red and purple) λ_{\parallel} and λ_{\perp} (A) demonstrate the significantly decreased λ_{\parallel} and increased λ_{\perp} in EAE mice. FTY720 treatment resulted in normalization of λ_{\parallel} and λ_{\perp} . Inflammation induced cellularity (tan; B) increase and edema (green; B) was normalized toward the control level after FTY720 treatment (B).

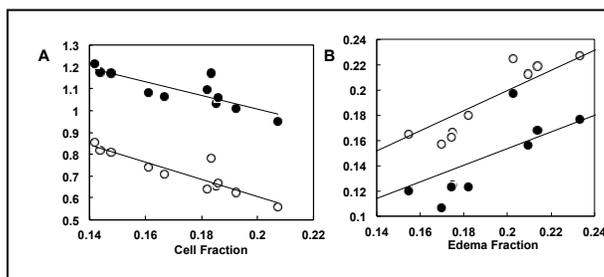


Figure 3. The effect of increased cellularity and vasogenic edema of EAE mice with and without FTY720 treatment was examined by scatter plots of (A) DTI (open circles) and DBSI (filled circles) derived axial diffusivity against cellularity, and (B) DTI and DBSI derived radial diffusivity as a function of edema fraction. Correlation analysis suggested that DTI derived axial diffusivity was more significantly correlated with cellularity than that of DBSI. Similar correlation was also seen between radial diffusivity and edema.