

Axonal Preservation of FTY720 Assessed Using In Vivo Diffusion Tensor Imaging

Xiaojie Wang¹, Joong Hee Kim², Jenet O'Neal³, Shawn O'Neal⁴, Joan Brieland³, and Sheng-Kwei Song²

¹Chemistry, Washington University, Saint Louis, Missouri, United States, ²Radiology, Washington University, Saint Louis, Missouri, ³Exploratory Immunobiology, Pfizer Inc., ⁴Investigative Pathology, Pfizer Inc.

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) with pronounced axon damage inflicting long-term neurological disability. In principle, the functional deficit induced by inflammation and demyelination may be reversible. In contrast, the disability resulting from the damage to axons and neurons is likely to be irreversible once the threshold of compensation is exceeded¹. Currently approved drugs for treatment of MS are primarily immunomodulatory agents with partial efficacy in relapse-remitting MS (RRMS) but modest to no efficacy in progressive disease². Recent studies have shown that decreased axial diffusivity ($\lambda_{||}$) and increased radial diffusivity (λ_{\perp}) derived from Diffusion Tensor Imaging (DTI) are specific and sensitive biomarkers of axonal damage and demyelination, respectively^{3,4}. Fingolimod (FTY 720, 2-amino-2-(2-(4-octylphenyl) ethyl)propan-1,3-diol), a novel sphingosine-1-phosphate receptor modulator recently demonstrated promise as a potential transformational MS therapy⁵. In this study, we employed in vivo DTI to evaluate the efficacy of both prophylactic and therapeutic treatments of FTY720 on experimental autoimmune encephalomyelitis (EAE) mice using $\lambda_{||}$, λ_{\perp} , and relative anisotropy (RA) as surrogate outcome measures.

Methods

Animal preparation: EAE was induced in C57BL/6 mice (N = 30) via injection of myelin oligodendrocyte glycoprotein (MOG) emulsified in incomplete Freund's adjuvant with *Mycobacterium tuberculosis* as previously described⁶. Control mice (N = 5 for each treatment group) received incomplete Freund's and *Mycobacterium tuberculosis* in the absence of MOG. Daily treatment immediately after MOG immunization (prophylactic) or at the onset of disease defined as clinical score ≥ 0.5 (therapeutic) with vehicle, 3 mg/Kg, or 10 mg/Kg FTY720 was carried out on EAE mice. Control mice received no treatments. **MRI:** Control and EAE mice underwent in vivo DTI examination on a 4.7 T scanner. A respiratory-gated multi-echo spin-echo diffusion weighted sequence was employed with actively decoupled volume (6-cm inner diameter, RF excitation) and surface coil (16 mm \times 9 mm, signal receive). The overall set up is similar to that described previously⁷. All procedures performed on these animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee. Images of 12 contiguous transverse slices covering T13 through L1 vertebrae segment were acquired using the following parameters: TR 1.2 sec (gated acquisition), TE 38 ms, Δ 20ms, δ 5 ms, slice thickness 1.0 mm, zero filled spatial resolution (38 μ m \times 38 μ m), total data acquisition time \sim 1.0 hr, (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0), (0,-1,1), and (1,0,-1), and b = 0 and 1.0 ms/ μ m².

Results

Representative in vivo DTI derived RA maps at the lumbar cord levels of the spinal cord from EAE mice undergoing prophylactic treatments clearly demonstrate that FTY720 preserves ventral-lateral white matter tracts (Fig.1). Similar protection effect was also observed in therapeutic treatments of EAE mice with axon and myelin integrity evaluated using $\lambda_{||}$, λ_{\perp} , and RA (Fig. 2). The decreased $\lambda_{||}$ and RA, and increased λ_{\perp} of the ventral-lateral white matter (VLWM) in lumbar segments of the spinal cord from EAE mice without treatment (group 4 of Fig. 2 A and B) were consistent with previously reported findings⁷. The end-stage values of $\lambda_{||}$, λ_{\perp} , and RA of lumbar segments of the spinal cord from EAE mice without treatment were 75% (indicating axonal injury), 156% (indicating demyelination), and 67% of the control values, respectively. There was no dose dependent treatment effect observed within each treatment group. Prophylactically (Fig. 2A), $\lambda_{||}$, λ_{\perp} , and RA values were 96%, 98%, and 99% of the normal for 3 mg/Kg; 93%, 107%, and 94% for 10 mg/Kg. Therapeutically (Fig. 2B), $\lambda_{||}$, λ_{\perp} , and RA values were 88%, 137%, 83% of the normal for 3 mg/Kg; 91%, 140%, and 83% for 10 mg/Kg. The prophylactic treatment showed a better preservation in both axon and myelin integrity than the therapeutic treatments of same dose. Significantly improved clinical scores were also seen in EAE mice treated with FTY720 (Fig. 3). However, there is no difference in clinical scores of EAE mice between prophylactic and therapeutic treatments (Fig. 3).

Conclusion

Both prophylactic and therapeutic treatments of FTY720 significantly improved clinical scores and preserved axon and myelin integrity. A better preservation of axon and myelin integrity of lumbar segments of spinal cord from EAE mice undergoing prophylactic treatment was observed in the current study, probably due to the early resolution of inflammatory insult reducing inflammation induced axon and myelin damages. Although the number of animals employed was still small, the preliminary finding suggest that in vivo DTI derived axon and myelin integrity markers may be used to evaluate the efficacy of disease modifying interventions in EAE and potentially MS.

References

1. Trapp et al., Ann Rev Neurosci, 2008. 31: 247-269.
2. Hemmer et al., Ann Neurol, 2007. 62: 314-26.
3. Song et al., Neuroimage, 2002. 17: 1429-36.
4. Song et al., Neuroimage, 2003. 20: 1714-22.
5. Cohen et al., N Engl J Med, 2010. 362: 402-15.
6. Budde et al., NMR Biomed, 2008. 21: 589-97.
7. Kim et al., Neurobiol Dis, 2006. 21: 626-32.

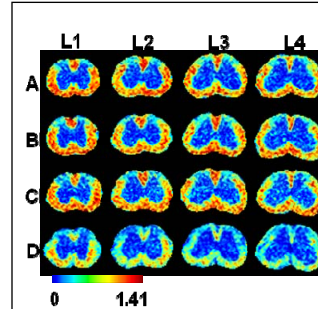


Figure 1. Representative relative anisotropy (RA) maps from prophylactic treatment group of EAE mice: (A) Control, (B) FTY720 3 mg/Kg, (C) FTY720 10 mg/Kg, and (D) EAE without treatment. EAE without treatment exhibits significantly lower RA indicating white matter injury.

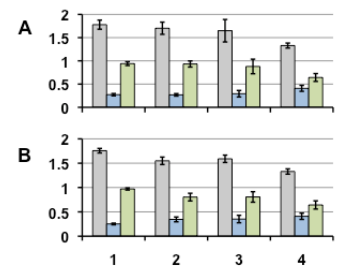


Figure 2. In vivo $\lambda_{||}$ (gray bars), λ_{\perp} (blue), and RA (green) of ventrolateral white matter averaged from L1 – 4 segments of control (group 1) and EAE treated with FTY720 at 3 mg/Kg (group 2), 10 mg/Kg (group 3), and vehicle (group 4) mice. The scale for $\lambda_{||}$ and λ_{\perp} is μ m²/ms; RA has no units. Mean \pm STD, N = 5.

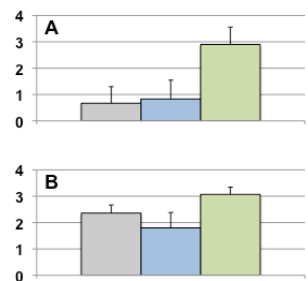


Figure 3. End stage clinical scores (CS) of EAE mice with prophylactic (A) and therapeutic (B) treatments at 3 mg/Kg (gray), 10 mg/Kg (blue) FTY720, and vehicle (green). The normal mice have CS of zero. Mean \pm standard error; N = 5.