Radial diffusivity Reveals a Role of Fibroblast Growth Factor 2: Inhibition of Remyelination after Chronic Demyelination

M. Xie[1], R. C. Armstrong[2], and S-K. Song[3,4]

[1]Department of Radiology, Washington University in St. Louis, St. Louis, MO, United States, [2]Department of Anatomy, Physiology, and Genetics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [3]Department of Radiology, Washington University, St. Louis, MO, United States, [4]Hope Center of Neurological Disorders, Washington University, St. Louis, MO, United States

Introduction

Multiple sclerosis (MS) is the most prevalent human CNS demyelinating disease. In MS, remyelination is limited, regardless of populations of oligodendrocyte progenitors (OP) in lesions. The OP is blocked to develop due to signaling molecules, including growth factors, cytokines and cell adhesion molecules in the lesions. Previous study[1] showed that absence of fibroblast growth factor 2 (FGF2) promotes OP differentiation into remyelinating oligodendrocytes and enhances remyelination. In this study, to monitor effect of FGF2 on remyelination non-invasively, *in vivo* DTI biomarker of myelin injury, radial diffusivity ($\lambda_\perp$)[2, 3], was measured longitudinally in cuprizone treated mice, a demyelinating animal model. The $\lambda_\perp$ increases in demyelinated axons and declines to original values as the axons are remyelinated. Although obvious increase in $\lambda_\perp$ was seen in both FGF2-/- and wild type (WT) mice following 12 weeks of cuprizone ingestion, only FGF2-/- mice showed significantly decreased $\lambda_\perp$ reaching close to control value after 12 weeks of recovery period suggestive of improved remyelination in FGF2-/- mice.

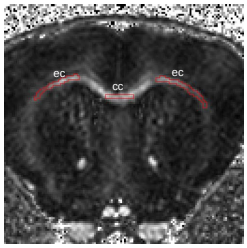

Figure 1. The regions of interest identified are shown on the RA map.

Materials and Methods

Animal Model

Eight week old male FGF2-/- mice (n = 3) were put on cuprizone diet (0.2% w/w of cuprizone; Harlan Teklad, USA) for 12 weeks. Subsequently, the treated animals returned to normal chow for 12 weeks. Age matched FGF2-/- mice (n= 3) on normal diet were used as the control.

Diffusion Tensor Imaging

Data were acquired using a standard spin-echo diffusion weighted imaging sequence. Acquisition parameters were: TR 1.7 sec, TE 50 msec, Δ 25 msec, δ 8 msec, NEX 4, slice thickness 0.5 mm, field-of-view 3 cm, and data matrix 256×256 (zero filled to 512× 512). Diffusion sensitizing gradients were applied along six directions: [Gx,Gy,Gz] = [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [0,-1,1], and [1,0,-1]. Two b-values (0 and 0.768 ms/μm^2) were used. Region of interest (ROI) was defined in corpus callosum (cc) and external capsule (ec) in DTI maps manually according to a mouse brain atlas. $\lambda_\perp$ was measured in cc and ec from 7 DTI slices encompassing the position from +1 to -2.5 mm of bregma (Fig. 1).

Results

To monitor the spatial and temporal progression of spontaneous remyelination throughout the course of recovery period, *in vivo* diffusion tensor imaging (DTI) was performed on live FGF2 -/- mice. Longitudinal $\lambda_\perp$ was collected in the ROI of cc and ec from each animal at four time points: 12 weeks of continuous cuprizone ingestion followed by returning to the normal chow for 6, 8, and 12 weeks. Volume-averaged $\lambda_\perp$ in cc or ec pooled from seven DTI slices were quantified (Fig.2 A and B). 12 weeks of cuprizone ingestion caused an increase in $\lambda_\perp$ by 44.3% (p = 0.002) and 20.2% (p = 0.008) in cc and ec respectively. $\lambda_\perp$ decreased during the recovery phase. After 12 weeks of recovery, $\lambda_\perp$ declined to close to the control level with only 19% (p > 0.1) and 9% (p = 0.1) higher than that of the control in cc and ec respectively.

Discussions and conclusions

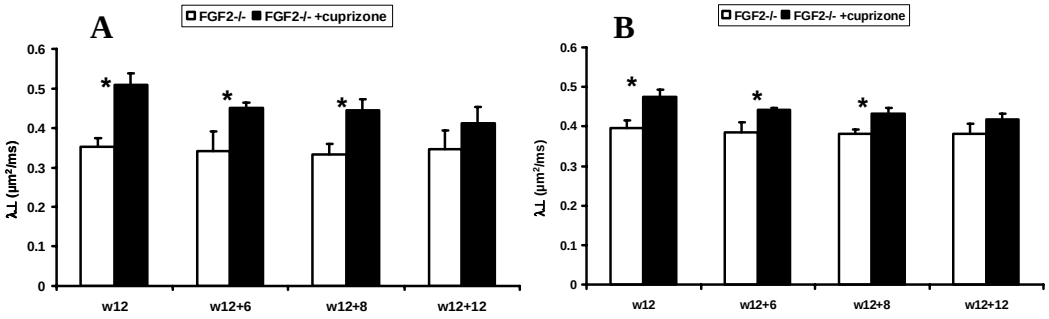

Figure 2 Comparison of volume-averaged $\lambda_\perp$ pooled from 7 DTI slices in cc (A) and ec (B) between cuprizone treated and non-treated FGF2-/- mice show a gradually declined trend of $\lambda_\perp$. w12: 12 weeks of cuprizone diet; w12+6, 8, and 12: 12 week – treatment followed by 6, 8, and 12 weeks of normal chow. *: P<0.05.

The cuprizone mouse model is characterized as a demyelinating and remyelinating animal model. Although 12 weeks of cuprizone ingestion induced chronic demyelination with limited remyelination in wt and FGF2 -/- mice, our previous study[3] and the current results demonstrated convincing remyelination during the recovery period evidenced by the normalized $\lambda_\perp$. In a previously conducted studies we observed that $\lambda_\perp$ is 56% (p = 0.003) and 31% (p = 0.009) higher than that of the control in cc and ec respectively in the WT mice at the end of the 12-week recovery following the 12 weeks of cuprizone feeding. In contrast, $\lambda_\perp$ stayed increased by 19% (p = 0.132) and by 9% (p = 0.110) compared to the control level in cc and ec respectively at the same recovery point. In addition, significantly increased oligodendrocyte density and myelin immunolabeling were observed 3 and 6 weeks of recovery after 12 weeks of cuprizone ingestion in FGF2-/- mice, but not in WT[1]. In summary, improved remyelination in the FGF2-/- mice was observed using DTI biomarker of myelin injury non-invasively. Therefore, DTI detection was a sensitive measure for *in vivo* detection of the improved repair of chronic demyelination.

References

1. Armstrong et al., J Neuropathol Exp Neurol 2006, 65: 245-256 2.Song et al., Neuroimage 2005; 26: 132-40.
3.Sun et al., Magn Reson Med 2006; 55: 302-8.