Increased radial diffusivity: A demyelination marker

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Introduction

Myelin damage, as seen in multiple sclerosis (MS) and other demyelinating diseases, impairs axonal conduction and is associated with axonal degeneration. Cessation of disease progression, promotion of remyelination and inhibition of axonal destruction are the focus of interventions. However, a diagnostic modality capable of non-invasively evaluating myelin integrity is currently not available to monitor the efficacy of new treatments.

Diffusion tensor imaging (DTI) is a newly developed technique that is capable of detailed characterization of water diffusion in central nervous systems (CNS). An analytical approach for interpreting DTI parameters that takes into consideration white matter structures and the underlying pathology has been proposed[1]. Evidence suggests that axial and radial diffusivities derived from DTI are directly linked to axon and myelin integrity[2].

To further test this hypothesis, we employed a well-established mouse model of reversible demyelination in the brain. The model involves the addition of cuprizone in the animal feed. It has been demonstrated that six weeks of cuprizone feeding results in severe to complete demyelination of the corpus callosum in the mouse brain[3]. In the present study, we used DTI to examine brains of control mice and mice administered cuprizone.

Materials and Methods

Animal preparations

Twenty-five eight-week-old male mice (C57BL/6) were divided into five groups of five animals each: 0-week, 3-week, 6-week, 9-week, and 12-week of 0.2% cuprizone feeding. The 0-week group mice are control mice that were not fed with cuprizone. The 9-week group was fed with cuprizone for 6 weeks followed by 3 weeks of normal chow. The rest of mice were fed with 0.2% cuprizone powder in the ground rodent chow according to the specified length. At the conclusion of each assigned length of feeding mice were deeply anesthetized and perfused, under anesthesia, through the left cardiac ventricle with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brains were refrigerated at 4°C in PBS until DTI examinations.

Diffusion Tensor Imaging

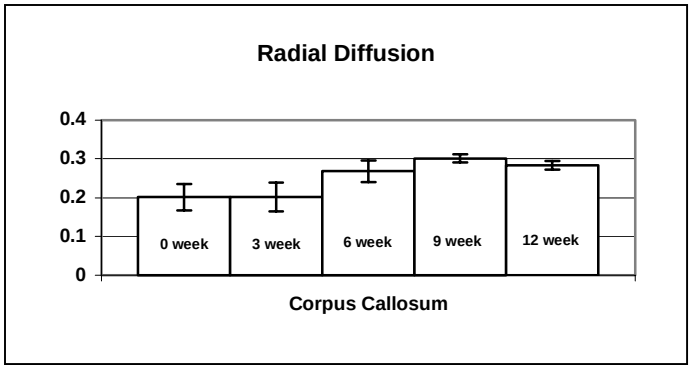
A conventional, multi-slice, spin-echo imaging sequence, modified by adding the Stejskal-Tanner diffusion sensitizing gradient pair, was employed for acquisition of the required series of diffusion weighted images (DWI). The *ex vivo* diffusion weighted images were acquired with repetition period (TR) 2 sec, spin echo time (TE) 35 msec, time between application of gradient pulses (Δ) 25 msec, diffusion gradient on time (δ) 10 msec, slice thickness 0.75 mm, field-of-view 1.5 cm, and data matrix 128×128 (zero filled to 256× 256). 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 diffusion-sensitizing factors, or b values (0 and 1.5 ms/μm^2) were used.

Data Analysis

The six independent elements of the diffusion tensor were calculated from each diffusion-weighted image. The resulting tensor element maps were used to derive the eigenvalues and eigenvectors of the diffusion tensor by matrix diagonalization. Three quantitative indices, including relative anisotropy (RA = normalized standard deviation of principal eigenvalues), axial diffusivity (λ_1), and radial diffusivity ($[\lambda_2 + \lambda_3]/2$), were derived using software written in Matlab (MathWorks, Natick, MA, USA)[1,2].

Results and Discussion

Elevated radial diffusion, suggesting myelin damage[1,2], was observed in corpus callosum of cuprizone fed mouse brains (Fig.). Cuprizone feeding is a well established model for investigating CNS white matter demyelination and remyelination processes. Thus, it is an ideal model to test the previously proposed interpretation of DTI parameters: increased radial diffusion as a marker of demyelination. Our current observation is consistent with the literature reports on mouse corpus callosum where significant demyelination was observed six weeks after 0.2% cuprizone treatment. The maximal elevation in radial diffusivity occurred after 6 weeks of cuprizone feeding ($p = 0.001$). There is no statistically significant difference in radial diffusivities among 6-week, 9-week, and 12-week groups. Thus the increased radial diffusivity is indeed parallel with corpus callosum demyelination.



References: [1]Song, et al., *Neuroimage*, **17**: 1429-1436 (2002). [2]Song, et al., *Neuroimage*, In Press (2003; available online). [3]Armstrong, et al., *J. Neurosci.*, **22**:8574-8585 (2002).