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High-Resolution Ex-vivo diffusion MRI revealed complex MS pathology in relapsing remitting EAE mouse model

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Introduction: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease where the patient symptoms vary according to the disease stage and the outcome of the treatments. MS neurodegeneration disease includes demyelination, axonal damage and nerve injury [1]. Various animal models have been developed to study these specific aspects of MS. Different MS models target specific questions. Experimental autoimmune encephalomyelitis (EAE); in particular, was designed to highlight the inflammation process that underlines the neurodegeneration. However the clinical disease pattern of EAE model is dependent on the immunization protocol that mostly exhibits chronic or secondary progressive disease without remissions recovery [2].

In this abstract, we describe the use of *ex vivo* high spatial and high angular resolution diffusion-weighted imaging (HARDI) to study the neuropathological changes in the EAE-mouse model of MS exhibiting remitting relapsing disease course with partial or complete recovery between symptomatic periods. This less aggressive approach could lead to a model that better reflects the pathobiological changes involved during early stages of MS.

Methods: EAE was induced in 4-6 weeks old C57BL/6 female mice by subcutaneous injection of MOG_{35-55} (200µg) emulsified in Saponin adjuvant (Quil-A) (45µg) and an intraperitoneal injection of pertussis toxin (PT) (250ng) as an adjuvant and to temporarily open the blood brain barrier. A second, identical injection of pertussis toxin was administered after 48 h. Sham mice received Quil-A and pertussis toxin only. Mice were monitored once daily over the 60-day experimental period for clinical scoring on a half-point scale ranged from 0 to 5.

MRI scanning of EAE (n= 7) and Sham (n=6) animals were conducted post-immunization during acute (3 sham and 3 EAE) (Day 13-16) and chronic (3 sham and 4 EAE) (Day 54-57) phases of the clinical course in comparison to naïve animals (n=4). Mice were anaesthetized and transcardially perfused with 0.1 M phosphate-buffered solution (PBS) followed by immersion of mouse heads in 4% PFA for 24 h. Brain tissues of animals were further washed with saline for 48h after extraction prior to MR imaging [4].

MRI data were acquired at 16.4 Tesla in a Bruker vertical bore animal MRI system using a 15 mm i.d. linear polarized volume coil. The 3D HARDI acquisition used the Stejskal-Tanner pulse-field gradient spin echo sequence, with 30 diffusion gradients directions at b = 3000 s/mm^2, δ/Δ = 2.4/6.4 ms, TR/TE = 400/14.5 ms, two b_0, at 100 micron isotropic resolutions. The total acquisition time was 14 h 44 mins. The FID data was zero-filled prior to a Fourier transform, resulting in 67micron isotropic resolutions.

Fractional anisotropy (FA) maps were calculated using the MRtrix program [5]. A FA map template was created from all datasets using the build template script of the program ANTS. Regions of interests (ROIs) were drawn manually on the template using ITK-snap at various white matter (WM) structures to check the extent of the demyelination [7]. The corpus callosum was divided into small segments, including forceps minor and major, rostral, middle and caudal. Other WM structures examined included the external capsule, Rt and Lt cerebral peduncles, Rt and Lt optic tracts and the internal capsule. ROIs were transformed back onto each subject using the FLIRT/FNIRT of the program FSL [6].

Results: EAE animals exhibited remitting-relapsing disease course with a mean score of 1.5 (limp tail and distinct hind limb weakness recognised by poor grip and unsteady gait) while sham control animals didn't reach to incidence of clinical disease**.** During acute phase, EAE mice exhibited a trend for FA reduction in many white matter structures, mostly affecting rostral, middle and caudal segments of the corpus callosum. However, FA values were not significantly affected in significantly chronic stages. Such changes, however, were observed both in the sham and the EAE animals, and were unrelated to the clinical scores (Figure1).

Discussion: The decrease in the FA observed in the sham animals may have resulted from a non-specific inflammation during the opening of the BBB by the PT, followed by Saponin. In the chronic stage, the FA values appeared to be recovering towards the control, which could indicate the process of repair (remyelination). This finding supports previous observations in that the diffusion tensor parameters are sensitive markers detecting subtle MS changes in the white matter [8, 9]. However, we also found that the high-resolution *ex vivo* DWI imaging was more sensitive in detecting such mild changes, because our studies performed on the same animal *in vivo* (not shown) were unable to distinguish this pathology.

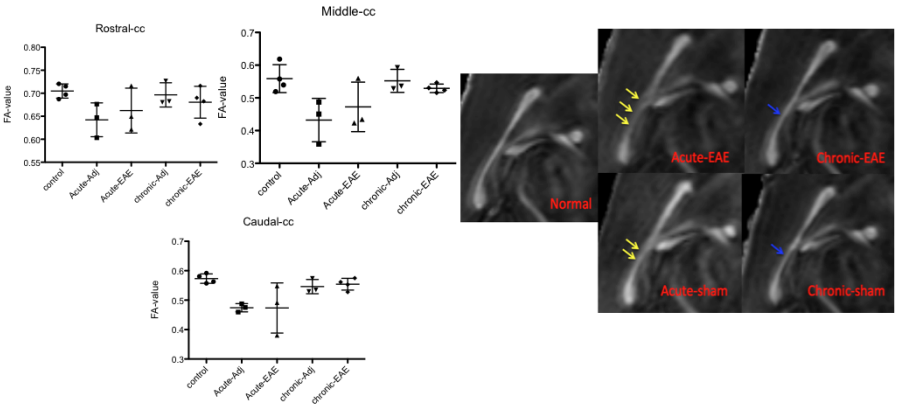

Figure 1: Reduction of FA in corpus callosum structures was observed from the rostral to the caudal segment. The FA reduction was stronger in the acute stage and appeared to recover towards normal in the chronic stage.

Conclusion: the DWI has been valuable in understanding the pathological process changes that occurred during the course of the relapsing remitting EAE. Future validation will need to be carried out using histology (for example, luxol fast blue) may confirm the presence of demyelination during the acute stages and spontaneous remyelination during the chronic stage, as suggested by the FA data.

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