Temporal Evaluations of the EAE-induced Pathologies in the Grey Matter of Chronic Relapsing EAE

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Synopsis: The present study aims in providing new insights into the mechanism of pathogenesis occurring during multiple sclerosis (MS) by utilizing experimental autoimmune encephalomyelitis (EAE), a well-accepted MS animal model. Complex pathology of MS is associated with the formation of plaques in the white matter and abnormalities in the grey matter during the disease attack. Temporal evaluation of the clinical and pathological disease progression was carried out using the conventional MR techniques along with localized proton MR spectroscopy (MRS) and contrast enhanced MRI. Our results demonstrated the temporal changes in brain structure and metabolites concentration before, during and after the EAE attack.

Introduction: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS. It is characterized by the two disease phases, the remitting-relapsing phase (RR phase) followed by the secondary progressive phase (SP phase). In spite of the wealth of studies for decades, the mechanisms involved in the disease progression and in the conversion from the RR phase to the SP phase remains largely unknown. Histological studies have proved the blood-brain-barrier (BBB) disruption, edema formation, inflammatory cell infiltration, demyelination and axonal damages to be the main pathological events occurring during the MS attacks. As the *in vitro* techniques are available only at one time point, the utilization of *in vivo* methodologies can provide information on the pathogenitic mechanisms, occurring during the disease emergence, the transient recovery, and during the disease relapsing phases. EAE mimics the human MS in several respects and is regarded as reliable model for MS. MRI allows *in vivo* assessment of several aspects of MS or EAE related pathological processes and is regarded as the gold standard for clinical evaluation of the disease status in MS patients [1]. As most of the research on MS is focused on the white matter lesions, there is growing evidence for the temporal changes occurring in the grey matter metabolite concentrations and their relationship with clinical impairments [2]. The objective of this study is to exploit conventional MR techniques along with localized proton MR spectroscopy and contrast enhanced MRI for delineating the disease-induced temporal changes in the EAE animal model of MS.

Materials and Methods: *Induction of EAE:* EAE was induced in female Lewis rats (8-10 weeks old) by active sensitization with spinal cord homogenate. The emulsion was injected into the tail base. Body weight and neurological aberrations were scored daily and graded from 0-4: 0 = normal; 1= loss of tail tonus; 2 = weakening of hind legs; 3 = paralysis of hind legs; 4 = paralysis of all legs.

MRI experiments: Experiments were carried out in 4 EAE rats. The animals were anesthetized using 5 % isoflurane and the anesthetization (1.5%) was maintained throughout the experiment. The body temperature and respiratory rate of the animals were monitored and maintained at 37^0C and 30-40 beats/min, respectively. All MRI experiments were performed on 4.7T Biospec 47/40 spectrometer with an active shielding gradient of 200 mT/m in 80 µs. All images were obtained in the axial orientation by using FOV = 3 cm, slice thickness = 1 mm and matrix = 256 * 128 zero filled to 256 * 256. T2WI images was obtained using multi-echo spin-echo sequence (TR = 4000 ms, TEs = 16, 32, 48, 64, 80, 96, 112, 128 ms, NEX = 1) and DWI images was acquired using Stejskal-Tanner spin-echo sequence (TR/TE = 1500/32.9 ms, NEX = 2, diffusion gradient duration = 7 ms, diffusion gradient separation = 15 ms, two b values = 0 and 1100 mm^2/s applied along X direction). Localized proton MR spectroscopy was performed on the brain stem region using PRESS sequence (TR/TE = 3000/136 ms, NEX = 256, Voxel size = 3.00 mm3). The peak areas of N-acetyl- aspartate (NAA), choline (Cho), creatine (Cr), lactate (Lac), and myo-inositol (Ins) were recognized and the ratios of brain metabolites relative to Cr were measured. Contrast-enhanced T1WI was performed using spin-echo sequence (TR/TE = 600/13.8 ms, NEX = 4) before and after a bolus I.V. injection of Gd-DTPA (0.15 mmol/kg, Magenevist, Germany).

Results: The EAE animals experienced a rapid decrease in body weight along with the increase in clinical score during the EAE attacks [Fig. 1]. The first EAE attack was observed from Day 9 to Day 18 and was followed by a recovery phase, while the second attack was observed from Day 23 to Day 28. The first attack evidenced significant enhancements of signal intensities in T2 and ADC maps of the brain stem region [Fig. 2 & 3] accompanied by the enlargement of ventricles. Data obtained from MRS delineated the rapid decrease in NAA concentration from the incitation of the first EAE attack and the decreasing trend continued throughout the course of the EAE attacks, which never returned to the normal concentration. In contrast, the Lac concentration was found to increase along with the increase in clinical symptoms of the first EAE attack and interestingly, was returned to normal after the incitation phase of the first EAE attack [Fig. 3]. No significant changes were observed in the Cho and Ins concentrations throughout the course of the EAE attacks [Fig. 4]. Gd-DTPA enhanced T1WI delineated that the damage occurring to the BBB from the start of the first EAE attack and the damage was persistent throughout the first attack, but no significant alterations in the Gd-DTPA enhanced signal intensities was found during the during the second attack.

Discussion: The signal intensity changes in the T2WI and DWI signify the edema formation occurring in the brain stem region. The intensity of edema formation and the enlargement of ventricles were found to be comparatively higher during the first EAE attack than in the first relapse, suggesting a higher degree of inflammatory cell infiltration during the first EAE attack. The permanent decrease in NAA concentration indicates the permanent neuronal dysfunction persistent throughout the disease course, while the increase in lactate concentration can be linked to the infiltration of T-cells and macrophages occurring during the first EAE attack. MRS was able to delineate the permanent damages occurring to the brain stem after the first EAE attack, while T2WI and DWI were unable to define any significant abnormalities during the recovery and relapsing phases. Consistent with previous reports [3], contrast-enhanced T1WI confirmed that the disruption of BBB occurs during the incitation of the first EAE attack and was not a significant feature of the second EAE attack. Our results underline the potential of temporal evaluations for the better understanding of the MS pathology which is of prime importance in the development of early diagnostic and therapeutic approaches.

References: 1. Rausch et al., (2003) Magn Reson Med. 50: 309-314. 2. Chard et al., (2002) Brain 125: 2342-2352. 3. Floris et al., (2004) Brain 127: 616-627.

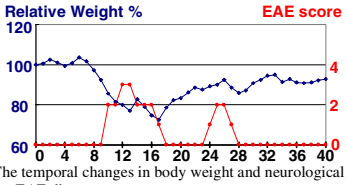

Fig. 1. The temporal changes in body weight and neurological deficits during the EAE disease course.

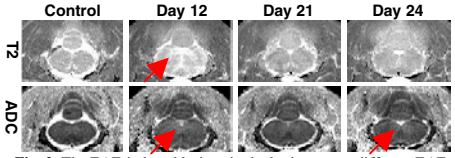

Fig. 2. The EAE-induced lesions in the brain stem at different EAE stages, revealed by T2WI and DWI. The upper set of images show T2 maps, while the lower set of images show ADC maps. Arrows indicate the regions with abnormal signal intensity.

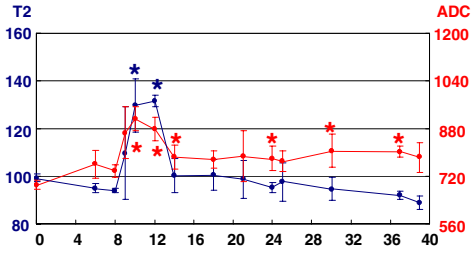

Fig. 3. The quantitative analysis of the temporal changes of T2 and ADC values in the brain stem. Dorsal brain stem near fourth ventricle was chosen as the ROI. Blue line corresponds to T2 values, while red line shows the ADC values. Significant changes in signal intensity are indicated by * in comparison to control. $P < 0.05$

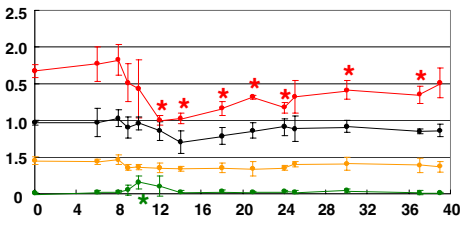

Fig. 4. The temporal changes of metabolites concentration in the brain stem region during the course of EAE attack, revealed by MRS. Red line indicates NAA/Cr. Black line corresponds to Cho/Cr. Yellow line show Ins/Cr. Green line corresponds to Lac/Cr. Significant changes in metabolite concentrations are indicated by * in comparison to control. $P < 0.05$.