Diffusion tensor imaging of $MOG_{34\text{-}56}$ and $MOG_{74\text{-}96}$ induced EAE in the common marmoset

G. J. Strijkers[1], E. Blezer[2], P. Smith[3], S. Amor[3], B. 't Hart[3], K. Nicolay[1]

[1]Biomedical NMR, Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Department of Experimental in Vivo NMR, Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands, [3]Department of Immunobiology, Biomedical Primate Research Center, Rijswijk, Netherlands

Introduction

Experimental autoimmune encephalomyelitis (EAE) in the common marmoset is a widely used and accepted model of chronic multiple sclerosis (MS). Two EAE models have been established in the common marmoset, which mainly represent the type II lesion pathology that is prevalent in chronic MS [1]. The first model uses immunization with myelin from sufferers of MS to induce the disease. The second model uses MOG (myelin/oligodendrocyte glycoprotein) to induce demyelination. Autoimmunity of marmosets to MOG has emerged as a common denominator in the immunopathogenesis of EAE in these models. The most important features of the myelin-induced EAE model can be reproduced in marmosets immunized with a recombinant protein that represents the extracellular N-terminal part of human MOG (1-125; rhMOG) [2,3]. The aim of this work was to investigate lesion formation in the common marmoset induced by two MOG epitopes, viz. $MOG_{34\text{-}56}$ and $MOG_{74\text{-}96}$. Both peptides emerged as dominant epitopes for T-cells from rhMOG-immunized monkeys. Immunizations with the individual peptides were performed in pairs of non-identical twins to analyze their contributions to the disease. *Ex vivo* T_2-weighted MRI and T_2-maps were used to characterize the local density of focal lesions and diffusion tensor imaging was used to assess possible differences in the severity of lesion demyelination.

Materials & Methods

Animals: EAE was induced in two groups of 4 non-identical twins in pairs by immunization with $MOG_{34\text{-}56}$ (N=4) and $MOG_{74\text{-}96}$ (N=4) as emulsion in CFA [3]. For ethical reasons the animals were sacrificed when an EAE score of 3 (hemi or paraplegia) was reached [3].

MRI: Experiments were performed *ex vivo* on a 6.3 T horizontal bore MRI scanner, equipped with a Varian VXRS imaging console and Magnex shielded gradients. The 4% phosphate buffered formaldehyde fixed brains were submerged in a perfluoropolyether (Fomblin, Ausimont, NJ) for susceptibility matching. Imaging parameters were as follows: FOV=2.5×2.5 cm^2, matrix=128×128, slice thickness=1 mm, number of slices=20. T_2-weighted images were collected using a spin-echo sequence with the following parameters: TR=4 s, TE=35 ms, NSA=8. T_2-maps were recorded with a multi-echo sequence with: TR=8 s, echo-spacing=20 ms, echo-train-length=8, NSA=4. Diffusion tensor images were made using a pulsed-field-gradient spin-echo sequence with: TR=4 s, TE=35 ms, NSA=8. Diffusion weighting was applied in 10 non-collinear directions with pulsed-field gradient parameters: Δ=20 ms, δ=10 ms, G_{diff}=0 and 120 mT/m, resulting in b-value=0 and 1717 s/mm^2.

Image analysis: First, the white matter (WM) was segmented manually on T_2-weighted images. In the WM, lesions were identified as regions with a T_2 value 10% above the normal appearing white matter (NAWM). Average T_2, apparent diffusion coefficient (ADC), and fractional anisotropy (FA) values were determined for lesions and NAWM for the 2 groups. Image analysis was done using Mathematica (Wolfram). Data (presented as mean ± SD) were evaluated by paired (siblings) t-tests. P<0.05 was considered statistically significant.

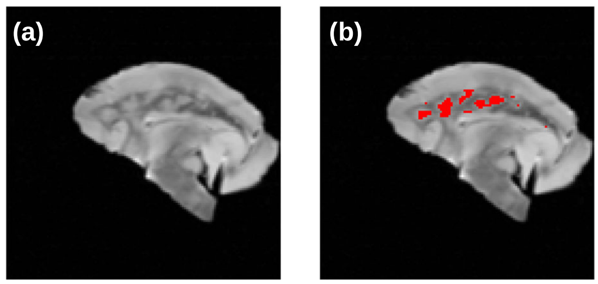

Figure 1: (a) Representative T_2-weighted image of marmoset brain with $MOG_{74\text{-}96}$ induced EAE lesions. (b) Segmentation of lesion area using threshold based on T_2.

Results & Discussion

Figure 1a shows a representative T_2-weighted image of the marmoset brain with $MOG_{74\text{-}96}$ induced EAE lesions. In figure 1b the result of the segmentation is presented. The hyperintense regions, which were histologically confirmed as lesions, could be segmented well from the surrounding NAWM. Lesions were identified in all of the marmoset brains. Figure 2 displays the average lesion density for the two groups as a function of slice position (average lesion density for $MOG_{74\text{-}96}$ = 20±4 % and $MOG_{34\text{-}56}$ = 14±3 %). The $MOG_{74\text{-}96}$ induction of EAE resulted in a significantly higher average lesion density, mainly anterior in the brains. In figure 3 the average differences in T_2, FA, and ADC of the NAWM and lesions are shown for the two groups of animals. T_2 and ADC in the lesions were significantly higher than in the NAWM. There was however no significant difference between the two groups. In both groups FA of lesions was increased compared to the NAWM. A significant difference was found in the FA of the NAWM, indicating a higher degree of demyelination of the $MOG_{74\text{-}96}$. No such difference was found, however, for the focal lesions.

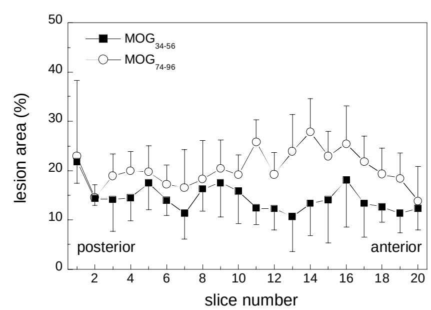

Figure 2: Lesion area in percentage of WM as function of slice position in the brain.

Conclusions

In summary we have used T_2-weighted MRI, T_2-maps, and diffusion tensor imaging to quantify EAE lesion formation induced by $MOG_{34\text{-}56}$ and $MOG_{74\text{-}96}$. Induction with $MOG_{74\text{-}96}$ resulted in a higher lesion density anterior in the brain and a significantly lower FA of the NAWM, indicating a higher degree of demyelination. These data show that the two MOG peptides cause differences in NAWM pathology and lesion distribution.

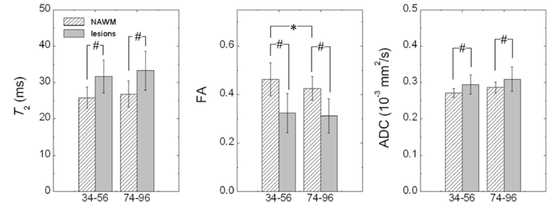

Figure 3: T_2, FA, and ADC for $MOG_{34\text{-}56}$ and $MOG_{74\text{-}96}$ induced EAE in NAWM and lesions (# lesions versus NAWM, * $MOG_{34\text{-}56}$ versus $MOG_{74\text{-}96}$, p<0.05).

References

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