Multicontrast MRI of re- and demyelination in the cuprizone mouse model

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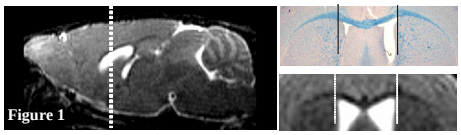
Introduction

Although magnetic resonance imaging (MRI) represents the most sensitive tool for the detection of white matter abnormalities in patients with multiple sclerosis (MS), the MRI correlates of specific pathophysiologic processes underlying MS lesion are largely unknown. Especially it is not clear how MRI reflects reparative processes such as remyelination. In order to identify probable MRI markers for de- and remyelination we took advantage of the cuprizone mouse model which allows a consistent induction and reversal of demyelination of the corpus callosum [1].



Methods

Adult male C57/BL6 mice (n=25) were treated with 0.2% cuprizone added to the food for 6 weeks. After withdrawal of the toxin mice (n=10) received a normal diet for additional 6 weeks. At 6 and 12 weeks after starting cuprizone treatment, three-dimensional T2 - and T1- weighted data sets were obtained with an isotropic spatial resolution of 117 µm [2]. Maps of the magnetization transfer ratio (MTR) were based on a spin density-weighted sequence with (M_{sat}) and without (M_0) off-resonance irritation [3]. MTR was given in percentage and calculated as $(M_0-M_{sat})/M_0$. All measurements were obtained at 2.35 T (Bruker Biospin). After MRI, the animals were sacrificed and histologically analyzed, including electron microscopy. Comparison of MRI and histologic data were performed in a transverse section as indicated in **Figure 1**. A quantitative region-of-interest analysis was based on MRI signal intensities in the lateral and medial part of corpus callosum normalized to the intensity of the cerebrospinal fluid (T1c, T2c). Age-matched animals (n=15) were included as controls. To evaluate the potential of different MRI contrasts to distinguish between different degrees of myelination, a discriminant function analysis was performed. The Wilk´s Lambda (λ) was used to evaluate the quality of group separation, which ranges from zero (best group separation) to one.

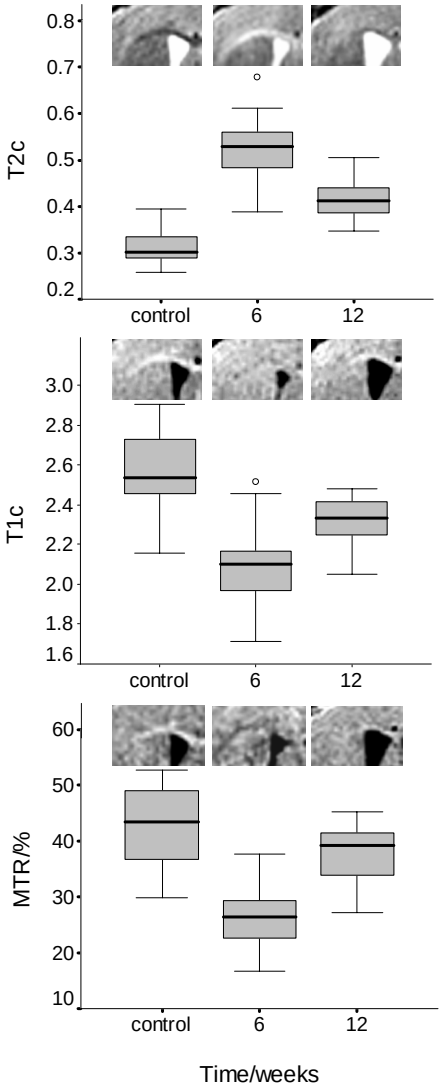


Figure 2*: Group analysis of the normalized signal intensities (T2c, T2c) and the MTR together with the corresponding MR images of controls, mice after 6 weeks of cuprizone treatment, and after additional 6 weeks of normal diet.*

Results

As shown previously [4] cuprizone treatment resulted in almost complete demyelination of the corpus callosum with only spare signs of inflammation and marginal axonal damage. MRI yielded a signal increase in T2- and a decrease in T1-weighted images as well as a reduction of MTR as demonstrated by quantitative group analysis in **Figure 2**. Withdrawal of cuprizone led to a substantial remyelination after additional 6 weeks of normal diet. The MTR level almost achieved the same value as obtained for untreated animals. In contrast, and despite a decrease of T2c and increase of T1c during remyelination, T2c and T1c values remained significantly different from those of controls. Comparing MRI results with electron microscopy revealed significant correlations of the diameter of axon divided by the fiber diameter as well as percentage of non-myelinated axons with T2c, T1c, and MTR. Although these findings confirm a direct pathophysiologic relevance of the observed MRI signal alteration, the group analysis of all three MRI contrasts showed considerable overlap between demyelination and remyelination as well as controls when considering T2c, T1c and MTR alone (**Figure 2**). Accordingly, discriminate function analysis predicted only 40% of the animals correctly using MTR, whereas T2c and T1c contrast alone correctly assigned 80% regions in the remyelinated group. The outcome was improved by a combination of two MRI contrasts. However, the most accurate prediction of myelin status was achieved by using all three contrasts yielding a correct assignment of 95% of all animals (λ = 0.089). The separation of groups is visualized in a 3D scatter plot of T2c, T1c, and MTR together with the results of discriminate analysis (**Figure 3**).

Conclusion

Multicontrast MRI allows for an improved distinction of demyelinated and remyelinated regions. This approach may considerably extend the proven value of MRI in MS and especially help in the assessment of novel myelin-targeted therapies in individual subjects.

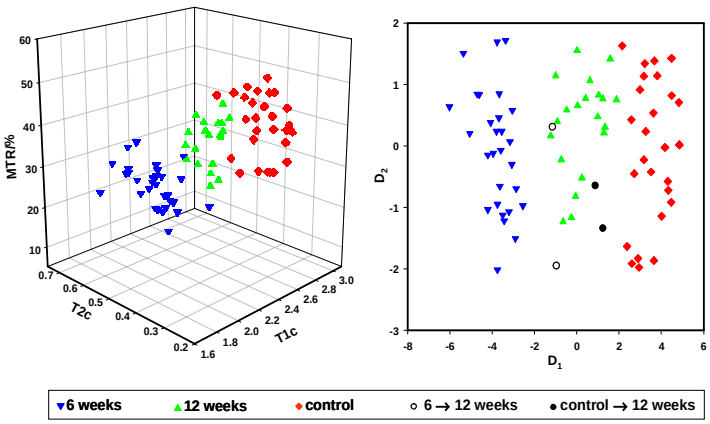


Figure 3: *Scatter plot (left) and discriminat function analysis (right) of normalized signal intensities (T1c, T2c) and the MTR in the lateral and medial part of corpus callosum of myelinated (control), demyelinated (6 weeks), and remyelinated (12 weeks) mice. Deviations between real and predicted group membership are indicated by white and black circles.*

References

[1] G.K. Matsushima et al, Brain Pathol. 11,107-16, 2001, [2] O. Natt et al, J Neurosci Methods., 120, 203-9, 2002, [3] O. Natt et al, Magn Reson Imaging., 211113-20, 2003, [4] S. Boretius et al, ISMRM, 12:1462, 2004