How do T1-, T2- and MT-weighted images reflect de- and remyelination? *In vivo* MRI of mice treated with the demyelinating neurotoxic agent cuprizone

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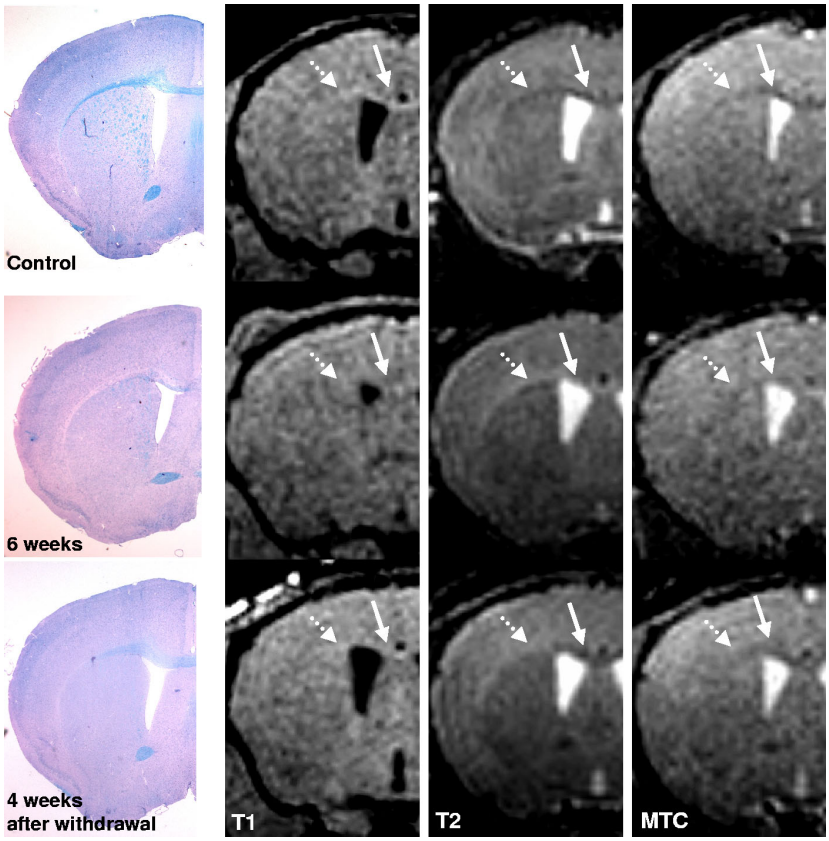
Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system. Histological studies of MS lesions have shown that remyelination can occur [1]. This may have significant implications for the improvement of health in MS patients. For the evaluation of therapeutic strategies targeting remyelination, monitoring of this repair process *in vivo* is essential. At present, magnetic resonance imaging (MRI) is the gold standard for MS diagnosis and the assessment of disease progression. However, it is not yet clear to which degree MRI reflects specific aspects of demyelination and remyelination. As a well-established animal model for de – and remyelination we used the copper-chelating neurotoxicant cuprizone administered to C57/BL6 mice for 6 weeks [2]. At different time points, T1-, T2- and MT-weighted images were obtained and compared with the corresponding histology.

Methods

Adult C57/BL6 male mice (n = 12) were fed with cuprizone (bis-cyclohexanone oxaldihydrazone) mixed into a standard powdered rodent chow (0.2% by weight) over 6 weeks. Afterwards they were returned to a normal diet for additional 4 weeks. Untreated age-matched animals (n = 4) were included as controls. At 3, 6, 9, and 10 weeks after starting cuprizone treatment T1-weighted (3 D FLASH, TR/TE = 17/7.58 ms, $\alpha = 25°$) and T2-weighted images (3 D FSE, TR/TE = 3000/98.25 ms, 16 echos, inter-echo-spacing = 12.5 ms) of 3 treated and 1 control animal were obtained, respectively. All measurements were performed at 2.35 T (Bruker, Biospin) at an isotropic resolution of 117 µm. MT-weighted images were based on a spin density-weighted 3D FLASH sequence ($\alpha = 5°$, TR/TE = 30/7.6 ms) with and without off-resonant RF irradiation (frequency offset: 5 kHz, mean amplitude: 200 Hz corresponding to a flip angle of 1045°). After imaging the animal were sacrificed and prepared for histology. Paraffin embedded, 1-2 µm thick sections were stained for myelin with luxol fast blue (LFB) and immunohistochemically with antisera against myelin basic protein (MBP) and proteolipid protein (PLP).



Results

Dependent on feeding duration cuprizone treatment resulted in a toxic demyelination with only very sparse signs of inflammation as shown by histology. This process was mainly confined to the area of the *corpus callosum* as illustrated in the LFB stained section of the 6 weeks time point compared to the control animal (first column, middle and top). The corresponding MR findings of the *corpus callosum* showed a reduction of the white matter signal in the control animal 6 weeks after cuprizone (second column). T2- weighted images (third column) revealed a corresponding signal increase. A similar observation was made in MT-weighted images (MTC, fourth column). The signal alteration can be interpreted as demyelination leading to corresponding increase of the extracellular water compartment.

The withdrawal of cuprizone resulted in remyelination as illustrated by LFB staining after additional 4 weeks of normal diet (first column, bottom). MRI at this time point revealed a slight signal increase in T1-weighted images (second column, bottom). The corresponding signal decrease in T2-weighted images seemed to depend on the degree of remyelination. However, a hyperintensive region was still detectable at 4 weeks after the end of cuprizone. The signal of the MT-weighed images also decreased without reaching normal values.

LFB-stained brain tissue sections in comparison with corresponding T1-, T2-, and MT-weighted images (MTC) of a healthy control mouse (top), after 6 weeks of cuprizone treatment (middle), and 4 weeks after withdrawal of the toxin (bottom). Contrast changes in the *corpus callosum* reflect areas of de- and remyelination as marked by arrows.

Conclusions

Similar to the observation in MS lesion signal losses in T1-weighted MRI and corresponding signal increases in T2- and MT-weighted MRI were detected in the cuprizone model. Here, these findings almost exclusively represent demyelination. Thus, this works demonstrates that contrast changes which are often interpreted as edema or cell infiltration may also reflect demyelination. With our mouse model, we can now elaborate MRI parameters reflecting the different stages of demyelination and remyelination. Such data may serve as a basis for *in vivo* differentiation of remyelinated versus demyelinated human MS lesions, which is essential for a validation and optimization of therapeutic regimens aiming at a remyelination of affected white matter in MS. Further studies will include a detailed quantitative analysis of the time course of MRI changes in comparison to histology.

References

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