Quantitative Magnetization Transfer Imaging of focal EAE lesions

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Summary

Quantitative magnetization transfer imaging was used to detect and characterize demyelination of white matter tracts in the internal capsule (IC) in a focal EAE model. Areas with reduced bound water fraction (20% lower than control values) were collocated with demyelinated axons of the IC. A change in T_2-relaxation of bound protons was not observed. In the case of mild demyelination, lesions appeared on the MT maps as diffuse areas of reduced MT suppression. Necrosis as found in some IC lesions led to a more pronounced focal reduction of the MT effect.

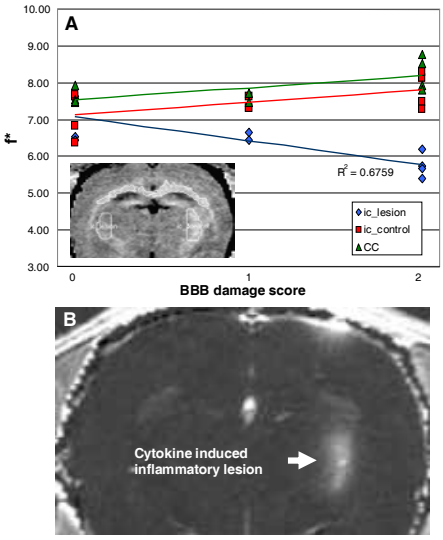
Introduction

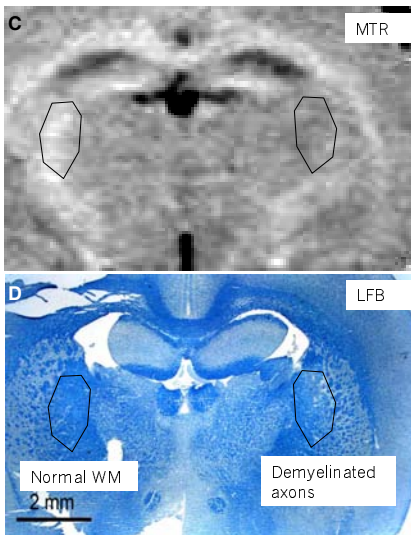
Demyelination and axonal loss are important hallmarks of multiple sclerosis (MS) since these are associated with neurodegeneration and long term neurological impairment. In order to characterize the pathogenesis of MS lesions and to test the neuroprotective effect of new or established drugs, it is very important to monitor demyelination and axonal loss non-invasively and over time. Magnetization transfer imaging (MTI) allows monitoring of molecular alterations within the tissue matrix and can be used to distinguish inflammatory from demyelinated MS lesions. However, the phenomenological and quantitative link between the character of the MS lesion and the MT signal is to a large extent unknown: most experiments have been carried out on humans, where the availability of biopsies is limited. Hence, it is difficult to determine the specificity or sensitivity of the method by direct comparison of MT data and histology. The aim of this work was therefore to compare quantitative MT (qMT) parameters with histology in an animal model of MS, in which reproducible white matter lesions were induced by focal cytokine injection.

Methods

To induce focal demyelination EAE lesions, female Lewis rats were immunized with a sub-clinical dose of Myelin Oligodendrocyte glycoprotein (MOG). 3 weeks later, focal lesions were induced by injecting 2 µl of a mixture containing 1.45 µg of TNF-α and 1 µg of IFN-γ unilaterally into the internal capsule using a glass capillary (N=7). Control animals were injected with the same volume of PBS (N=4).

qMT: All measurements were carried out on a 7T PharmaScan System (Bruker Biospin, Ettlingen, Germany). A cylindrical quadrature coil was used for transmission and reception. 3D-FLASH datasets were recorded with six different offset frequencies: Δ=500, 1000, 2523, 6339, 15924, 25000Hz. The parameters of the Gaussian MT-pulse were: pulse length=14.6msec, FWHM=187.7Hz, pulse angle=1050rad s^{-1}. Determination of MT parameters (fraction of bound water f* and T_{2b}) were carried out as described by Tozer et al. (Tozer et al., 2003). T_1 was fixed in this model to 1450 msec because the lesions were not visible on the T1-weighted images and because the model is relatively insensitive with respect to changes of the longitudinal relaxation time: a change of T_1 from 1500 to 2000msec led to a change in f* of around 2.5%. Gd-enhanced MRI was used to confirm and determine the localization of the inflammatory lesion. The grade of BBB damage was scored according to the following scale: 0=no enhancement, 1=slight enhancement with diffuse boundaries, 2=clear enhancement. ROIs were defined for the internal capsule (IC) on the lesion and control hemisphere. Another ROI was defined for the corpus callosum (CC). Brains were fixed in formalin and sections were stained with Luxol fast blue and H&E.





Results and Discussion

Cytokine injection led to a reproducible inflammatory brain lesion in the target area (H&E stain) and extensive demyelination of the IC (LFB stain). Clear BBB damage was observed only in the hemisphere where the cytokines were injected (grade 2). Grade 1 enhancement was observed also in two PBS animals. This mild BBB damage might have been induced mechanically by the tip of the glass capillary. Qualitative MTR images of these lesions showed areas of decreased MT effect in the IC of the lesion hemisphere of cytokine-injected animals. No signal abnormalities were detected in the control hemisphere or for the CC. Quantitative analysis revealed that the amount of bound water (f*) in the IC was reduced by around 20% from 7.2±0.7 pu sec (mean±stdev) in normal tissue to 5.7±0.3 p.u. sec for IC lesions, which were clearly visible on the Gd-enhancement maps (Figure 1,B) . No lesion specific effect was observed for T_{2b}. Areas of reduced f* corresponded to tissue with demyelinated axons but not necessarily axonal loss. f* of a necrotic part of a lesion was further reduced to 4.23 p.u. sec. Hence, the qMT method allows direct quantification of demyelination as one of the most important pathological processes in MS or EAE. This will allow to better understand lesion development in EAE or MS and to study demyelination, remyelination and neuroprotection non-invasively for new treatment approaches in MS. Since T_{2b} didn't change in lesions it must be assumed that demyelination does not affect the binding of water molecules to the myelin sheath or other membrane elements. This would potentially allow to keep T_{2b} constant in the model, which would make the modeling process even more robust and might allow to analyze datasets with lower SNR or to use smaller ROIs.

References

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