In-vivo quantitative magnetization transfer imaging of de- and re-myelination in cuprizone-treated mice and correlation with histology

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TARGET AUDIENCE: Neuroscientists with an interest in the development and validation of MRI biomarkers of myelin.

PURPOSE

Quantitative magnetization transfer (MT, [1]) is an of extension MT imaging enabling the estimation of a number of properties of the macromolecular protons in proteins and lipids. It is often assumed that in the white matter of the brain the MT process is dominated by myelin. Consistent with this hypothesis, clinical studies in patients with multiple sclerosis have indicated that quantitative MT parameters can differentiate clinical phenotypes and closely correlate with the severity of clinical symptoms [2]. Previous animal studies [3,4] suggest that one of these parameters, the macromolecular pool ratio (F), is an indirect measure of myelination. However, these studies were conducted ex-vivo, following perfusion and complete tissue fixation, and therefore may not represent in-vivo conditions. Addressing this limitation, here we examined cuprizone-treated mice (a reversible model of demyelination,[5]) with in-vivo quantitative MT and subsequent histology.

MATERIAL AND METHODS

Experimental model All procedures complied with directive 86/609/EEC and were part of a project approved by the Italian Ministry of Health (ref. 31/2011-B). Six-week old C57BL/6 male (n=15) and SJL/J female (n=15) mice were obtained. After 1 acclimation week, 10 mice for each strain were fed cuprizone (0.2% w/w) for 5 wks. (C57BL/6) or 7 wks.(SJL/J) to obtain robust demyelination. The remaining 5 received regular chow diet. At 5/7 wks., 2 control and 2 treated mice were submitted to histology, 4 returned to normal diet and 4 continued receiving cuprizone for a total period of 10 wks. Mice were euthanatized through deep anesthesia; tissues were fixed with 4% PFA in PBS.

Imaging MRI was performed at baseline (0 wks.), treatment completion (5/7 wks.) and experiment end (10 wks.), using a Bruker Biospec 70/30 system. Two coronal sections, perpendicular to brain base and positioned on the genu and splenium of the corpus callosum (0.75 and -1.35 mm to the bregma) were studied.MT-weighted images were acquired using a FLASH sequence (TE=3.3 ms, TR=45.4 ms, NEX=8, thickness 600 um, 0.1 mm^2 in-plane resolution). The MT pulses were Gaussian and had excitation angles 1000 and 4000 deg, and offset frequencies ranging between 1 and 30 kHz. T1-weighted images were acquired with a RARE sequence having identical geometry, TE=9.2 ms, and TR ranging between 200 and 8000 ms. Following T1 estimation, F maps were obtained by fitting voxel-wise, a modified model equation for the MT-weighted signal using the Levenberg-Marquardt method [2].

Histology Coronal slices at 0.75 and -1.35 mm to the bregma were cut from frozen tissue. Histological myelin staining was performed using Black Gold II on 20 µm brain sections; immunofluorescence was performed using anti-MBP primary antibody, followed by Alexa Fluor 488-conjugated goat anti-rabbit secondary antibody on 10 µm brain sections. Images were captured using a confocal laser-scanning microscope (40xmagnification).

Data Analysis Regions-of-interest were drawn over the medial and lateral corpus callosum. F-value was calculated, alongside pixel intensity ratio with respect to background for histology. Values from the medial and lateral regions and from 2 independent operators were averaged together. Comparisons were performed using Mann-Whitney-Wilcoxon tests, and F measurements were correlated with histological measurements using linear regression.

RESULTS

Cuprizone-induced demyelination, and remyelination following treatment suspension, were evident for both strains, as confirmed by Black Gold II histology and MBP immunofluorescence. F-values consistently indicated demyelination at the first treatment time-point with respect to baseline, with a significant aggravation at the second treatment time-point for both brain sections and strains (Fig 1). For C57BL/6 mice only, significant remyelination was evident in the subgroup for which treatment was interrupted after the first time-point. Pooling the time-points and brain sections together, strong linear relationship was found between F and Black Gold II for C57BL/6 mice (r^2=0.4427) and SJL/J mice (r^2=0.5408). Significant correlations between F and MBP were observed for C57BL/6 mice (r^2=0.4854) and SJL/J mice (r^2=0.2866), with clear group clustering. Interestingly, weak correlation was found between F and β-tubulin for C57BL/6 (r^2=0.3877), but not for SJL/J mice (r^2=0.0005442), suggestive of neuronal fragility induced by damaged myelin shealths, in the more susceptible murine strain.

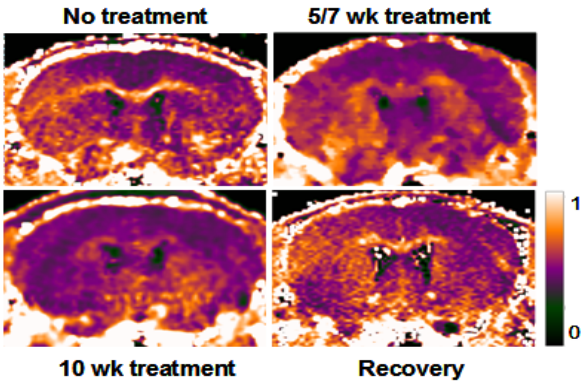

Fig 1. F maps from a C57BL/6 mouse. The change in contrast between the corpus callosum and the surrounding grey matter can be appreciated from baseline (top left), through demyelination (top right and bottom right), and remyelination (right).

DISCUSSION

The finding of significant F differences in cuprizone-treated mice corroborates previous ex-vivo work on shiverer mice in demonstrating that this parameter is directly linked to myelin status [3,4]. Here, this relationship was further confirmed through the observation that the spontaneous remyelination which ensues upon treatment suspension was promptly detected in C57BL/6 mice as an increase in F. Our results provide the first direct confirmation that F estimated from quantitative MT imaging performed in-vivo is a viable proxy of myelin status, showing a strong linear correlation with histological myelin staining.

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