

Regional assessment of Magnetization Transfer Ratio in the mouse brain - tracking myelin change in a model of toxic demyelination.

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Introduction: In demyelinating disorders of the central nervous system, the possibility of assessing myelin content quantitatively in the brain would be an important tool for evaluation of disease activity and treatment efficacy. Myelin content cannot be assessed using conventional MR techniques, due to its low T2 relaxation time. Magnetization Transfer Ratio (MTR) is a semiquantitative method for assessing myelin content. In this study, we have

developed a guided, semi-automatic method for determining mean MTR in different regions of the mouse brain. By comparing MTR values to histopathology from an animal model of toxic demyelination, the cuprizone model, we will investigate the relation between myelin- and MTR change.

Methods: Animal model and histopathology: Demyelination was induced in 48 eight week old female C57B/6 mice by adding cuprizone 0.2% w/w to the mouse chow for six consecutive weeks. All mice, including a control group of six healthy age- and gender matched mice, were MRI scanned at baseline. The control mice and six of the cuprizone exposed mice were scanned weekly throughout the whole period of cuprizone exposure, and two weeks after ending cuprizone exposure. Each week during cuprizone exposure six mice were scanned and thereafter sacrificed for histopathology. Formalin fixated, paraffin embedded coronal sections from +/- 1 mm from the bregma were histochemically stained for myelin by Luxol Fast Blue (LFB) - Cresyl Violet. The myelin content in the midline of corpus callosum (CC-med), laterally in the corpus callosum (CC-lat) and in the deep gray matter (DG) was scored semiquantitatively by an experienced blinded observer (SW). All experiments were conducted according to FELASA guidelines, and approved by the Norwegian Authority on Research in Animals. **MRI:** MRI images were acquired using a 7-T horizontal bore magnet (Pharmascan 70/16, Bruker BioSpin). The mice were anesthetized by isoflurane 1.5% in O₂ / N₂O₂ (60% / 40%). During scanning, the body temperature and respiratory frequency were monitored. **M0:** 3D Fast Low Angle Shot (3D-FLASH - Gradient echo sequence), 30x128x128 matrix size, 8 averages, 2.56 x 2.56 x 0.7 cm³ FOV, 0.2x0.2x0.23 mm³ resolution, TE 2.3 ms, TR 28.5 ms, 10° flip angle, 10 minutes. **Ms:** The same as M0, but with a magnetization transfer preparation pulse (+2500Hz off resonance, Gaussian shaped, 7.5μT, 15 ms). **T2:** Rapid Acquisition with Relaxation Enhancement (RARE - Spin echo sequence), 30x128x128 matrix size, 2.56 x 2.56 x 0.7 cm³ FOV, 0.2x0.2x0.23 mm³ resolution, TE 9 ms, TR 1500 ms, RARE factor 16, 6 minutes. **Image analysis:** Full brain segmentation was obtained using a guided semi-automatic method, whereby an experienced operator generated a complete segmentation map of one brain, this map was then superimposed on all other images using coregistration. Mean MTR value was calculated in each segment.

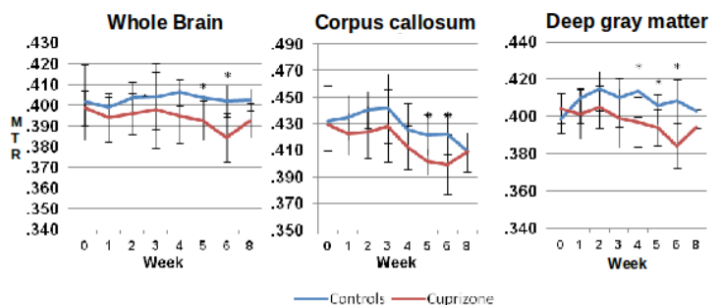


Figure 1. Mean MTR values in whole brain, corpus callosum and deep gray matter at different durations of cuprizone exposure. Error bars are 1 SD. * $p < 0.05$, independent samples t-test.

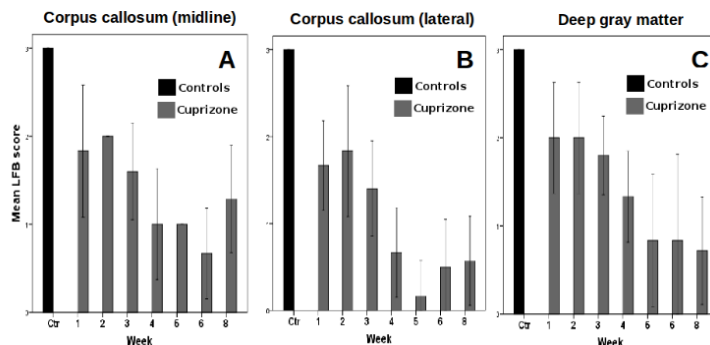


Figure 3. Semiquantitative myelin scores evaluated in LFB-stained sections from the midline of (A) and lateral (B) corpus callosum, and in the deep gray matter (C). 0 equals complete demyelination, while 3 equals normal myelin status. Error bars are 1 SD.

Results: A significant reduction in MTR values was detected in the whole brain and in the deep gray matter after 4, 5 and 6 weeks of cuprizone exposure ($p < 0.05$, independent samples t-test). In the corpus callosum, significant reduction in MTR values was detected after five and six weeks, respectively ($p < 0.05$, independent samples t-test) (Figure 1, 2). In the LFB-stained sections from the corpus callosum, loss of myelin integrity was observed after one week of cuprizone exposure ($p < 0.0005$, One-way ANOVA), (Figure 2, 3).

Discussion and conclusion: In this study, we show that tracking MTR changes during cuprizone-induced demyelination using a guided, semi-automatic is a feasible way of monitoring myelin loss. Detecting significant changes in deep gray matter after four weeks of cuprizone exposure is in agreement with what is known about the temporal dynamics of the cuprizone model. In the early periods of cuprizone exposure, the histopathological changes observed is first and foremost structural changes in the myelin, not quantitative. The MTR will not distinguish between viable, functional myelin and myelin debris, which is a possible explanation for the failure of MTR to detect early changes compared to histopathology. In conclusion, using segmented region-maps may be a promising method for in-vivo assessment of demyelination in the cuprizone model and in MS patients as well.

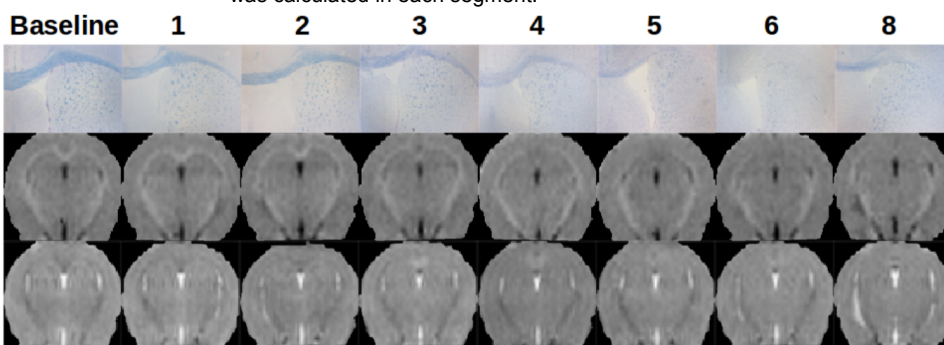


Figure 2. LFB stained coronal sections of mice brains, taken from +/- 1mm from the bregma (top). MTR- (middle) and T2 (bottom) from weekly scans of a cuprizone exposed mice.