High field T1 predicts neuronal loss in multiple sclerosis cortical grey matter

K. Schmierer1, P.-W. So2, S. F. An3, S. Brandner1, D. H. Miller1, T. A. Yousry4, and H. G. Parkes1


INTRODUCTION: In multiple sclerosis (MS) brain white matter (WM) magnetisation transfer ratio (MTR) and T1 are associated with myelin content and – to a lesser degree – axonal count (1). The substrate of these MR indices in MS cortical grey matter (CGM) is less clear. In this study we investigated the association between myelin content and neuronal density (ND) with T1, T2 and MTR in MS CGM using high-field MRI and quantitative histology.

METHODS: Post mortem brain from eight women and four men with MS was used for this study. The patients’ age at death was 54 years (SD 10 years), disease duration 23 years (7 years), and expanded disability status scale (EDSS) score 8 (median 8.75). Brain samples had been fixed in 10% formalin for 1090 days (SD 486 days). Post mortem interval was 53 hours (35 hours). Coronal brain slices were provided by the UK MS Tissue Bank, Imperial College London, and dissected at the level of the internal capsule. Tissue blocks from these slices were fitted into histology cassettes, inserted into a universal tube, immersed in perfufluoropolyether, and placed in a quadrature H volume MR coil (24 mm diameter) for scanning on a 9.4T Varian Inova system. MR experiments included spin echo acquisitions with TR/TE [ms], 480-4000/16 and 20/24-60 for T1 and T2, respectively. Field of view was 30 x 30, matrix size 256 x 192 (2 averages). For MTR maps gradient-echo acquisitions were collected using TR/TE 186/5, FOV 30 x 30; matrix size 256 x 256 (16 averages) with and without RF saturation pulses at 6 and 100kHz offset from water resonance. T1, T2 and MTR maps were produced by fitting signal intensities to the appropriate equations on a pixel-by-pixel basis using ImageJ (NIH, Bethesda, USA). After scanning tissue blocks were re-immersed in formalin prior to processing for embedding in paraffin. Sections were (immuno-) stained for myelin-basic protein (myelin) and cresyl-violet (CV). Regions of interest (ROI) were identified on the MR images and categorized into healthy looking cortex (HLC) and cortical grey matter demyelination (CGML). CGML were classified according to established criteria into types I (juxtacortical), II (intracortical), III (subpial, not reaching the WM), and IV (subpial, reaching the WM GM border) (2).

Myelin content was quantified by measuring transmittance on MBP stained sections (3). Values obtained were expressed as inverse transmittance (1/transmittance, iTrans). Neurons were identified on CV stained sections according to morphological criteria (4), and quantified by unbiased univariate sampling, established criteria into types I (juxtacortical), II (intracortical), III (subpial, not reaching the WM), and IV (subpial, reaching the WM GM border) (2). Myelin content was quantified by measuring transmittance on MBP stained sections (3). Values obtained were expressed as inverse transmittance (1/transmittance, iTrans). Neurons were identified on CV stained sections according to morphological criteria (4), and quantified by unbiased univariate sampling.

RESULTS: Nineteen CGML (nine type I, four type II, five type III, one type IV) were analysed. HLC and CGML differed in T1, MTR and ND whereas only (figure 2), (iii) MTR and myelin content (figure 3), and (iv) T2 and ND. Marginal significance emerged for an association between iTrans and ND. Duration of fixation was strongly associated with MTR (r=0.76; p<0.01), T1 (r=0.62; p<0.01), ND (r=0.44; p=0.01), and iTrans (r=-0.55; p=0.01), however not with T2 (r=0.03; p=0.88).

CONCLUSION: Despite the fact that the cortex contains much less myelin than the white matter, MTR appears mainly dependent on myelin, at least in chronic post mortem MS cortex, whereas T1 emerged as the strongest predictor of ND. The modest association between ND and myelin content along with a loss of only ~20% neurons in our sample of chronic MS brains (similar with ref 4) suggests that neurons and/or their environment may be better equipped than axons against the consequences of the damage MS causes. The difference compared to 50% (or more) axonal loss in MS white matter lesions remains striking. Our data further suggest that fixation time is an important confounder that needs to be taken into account in studies using fixed post mortem samples.


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TABLE 1 NAC (SD) CGML (SD) p
T1 [ms] 730 246 718 293 0.7
T2 [ms] 23.8 4.9 27 5.1 <0.01
MTR [pu] 33.9 7.1 27.5 9.5 <0.01
Neurons/mm2 139 50 115 42 <0.01
iTrans (myelin) 1.41 0.27 1.35 0.24 0.2

TABLE 2 T1 MTR T2 iTrans (myelin)
MTR = 0.84
p<0.01
T2 = 0.19 r=0.07
p=0.28 p=0.77
iTrans (myelin) = 0.31 r=0.44 r=0.22
p<0.01 p=0.04 p=0.22
Neurons = 0.48 r=0.3 r=0.39 r=0.35
p<0.01 p<0.17 p=0.02 p=0.04

FIGURE 1 Myelin basic protein
High resolution
Type III lesion Type I lesion

FIGURE 2 Myelin (iTr) vs MTR

FIGURE 3 Neuronal density vs T1

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