INTRODUCTION

Multiple sclerosis (MS) affects the phosphorylation and thus the proton binding capacity of axonal neurofilament (Nf) proteins (1). Macromolecules can be quantified by measuring the exchange of magnetization between protons in the semisolid fraction of the tissue and protons in the mobile fraction. Using post mortem brain we explored whether in MS non-lesional white matter (NLWM) magnetization transfer ratio (MTR) is associated with Nf phosphoforms, thus potentially providing a biomarker of axonal phosphorylation in NLWM.

METHODS

Unfixed post mortem brain slices of 10 women and 2 men (age: 56 years, standard deviation [SD] 14 years) with MS for 23 (SD: 10) years were used (table). Brain slices were retrieved 17 (SD: 6) hours after death and small snap frozen samples (n=128) of macroscopically NLWM obtained, homogenised, and protein levels quantified using enzyme linked immunosorbent assay (ELISA) for Nf heavy chain (NfH) phosphoforms, glial fibrillary acidic protein (GFAP), S100B and ferritin. MRI of the remaining tissue was performed 52 (SD: 23) hours post mortem on a Signa Horizon Echospeed 1.5T system to acquire (i) 2D dual spin-echo (SE) proton density (PD)- and T2 weighted (TR: 2000 msec; TE1/TE2: 30/120 msec; flip angle: 90°; matrix size: 256 x 192 reconstructed to 256 x 256 over a field of view [FOV] of 24 x 24 cm²), (ii) 2D PD- and T1 GRE (TR/TE/flip angle: 1500 msec/11 msec/45° and 36 msec/11 msec/45°, respectively), from which T1 maps were generated, and (iii) 2D dual SE images (TR/TE/flip angle: 1720 msec/80 msec/90°) obtained with and without a saturation prepulse (16 msec, 23.2 µT Hamming apodized three-lobe sinc pulse, applied 1 kHz off water resonance) from which MTR maps were generated. Matrix size for the T1 and MTR maps was 256 x 256, and the FOV was 240 x 240 mm² (2) (fig 1). After scanning, tissue blocks were dissected, processed for embedding in paraffin, and sections stained for Luxol-fast blue (myelin) and Bielschowsky’s silver impregnation (axons) (fig 2).

RESULTS

Hyperphosphorylated NfH-SMI34 – but not total NfH-SMI35 – levels varied between individual patients’ NLWM. Concentration of hyperphosphorylated NfH-SMI34 correlated with T1 (r=0.7; p= 0.01) and inversely with MTR (r=-0.76 p<0.01) (fig 3), whereas NfH-SMI35 did not correlate with any MRI index.

CONCLUSION

Post-translational modifications of axonal proteins such as phosphorylation and compactness of NfH are early signs of axonal damage. The association between hyperphosphorylated NfH (i) T1 and (ii) (inversely) MTR suggests that early axonal changes on a proteomic level may be detectable in MS NLWM in vivo.

REFERENCES


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