Detection of Axonal Damage in the Spinal Cord in Multiple Sclerosis by Magnetic Resonance Spectroscopy

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Introduction

Diffuse axonal damage is a well recognised component of multiple sclerosis (MS) and can be robustly evaluated in the brain using proton magnetic resonance spectroscopy (MRS)[1]. Histological examination of pathological specimens has also confirmed that significant axonal loss occurs in the spinal cord in MS[2,3]. As these studies used *port-mortem* samples, they do not provide any detail about the progression of axonal loss or its relationship to clinical symptoms. Recently we have described a protocol for obtaining proton MR spectra from the spinal cord in normal volunteers[4]. In this current work we apply this technique in a cohort of patients with MS to investigate the relationship between clinical function, MR imaging changes and axonal damage in the spinal cord and brain (as defined by N-acetyl-aspartate – NAA – levels using MRS).

Methods

Patients: Eleven patients with multiple sclerosis (mean age 44±9 years, 4 female, 4 relapsing remitting, 7 secondary progressive) were recruited from the local neurological service and compared with 12 healthy controls (mean age 37±12 years, 4 female). Clinical assessment of patients included full history, EDSS, timed walk over 10m and 9 hole peg test. All investigations were approved by the local ethical review committee.

MR Protocol: Subjects were studied in a 2T whole body spectrometer with a Bruker Avance console (Bruker Medical GmbH, Ettlingen, Germany). Proton spectra were collected from 2 voxels – 1 in normal appearing fronto-parietal white matter and 1 in cervical spinal cord. Following sagittal and transverse T1 weighted imaging (0.7 mm in-plane resolution) of the spinal cord, spectra were collected from a 9×7×35mm^3 voxel located at the level of C3 using a purpose-built quadrature surface coil and according to our previous protocol[4] (cardiac gated PRESS sequence, TR=3s, TE=30ms, 256 averages). Subjects were then removed from the magnet and the coil exchanged for a head birdcage coil. Following transverse T2 weighted imaging (TSE sequence, TR=3s, TE=80ms, 20×5mm thick contiguous slices, 1 mm in-plane resolution), a single voxel spectrum was acquired from a 2×2×2 cm^3 voxel in normal appearing fronto-parietal white matter, taking care to exclude any areas of hyperintensity from the selected voxel (PRESS, TR=3s, TE=30ms, 128 averages). In both MRS voxels, water data were collected for absolute quantitation using a fully relaxed (TR=10s) multi-echo sequence with echo times varying from 35 to 2235 ms.

Data Processing: Brain images were analysed by image segmentation to assess total volume of T2w hyperintense lesions. Spinal cord images were used to determine cross sectional area of the cervical cord at the level of C3. Proton spectra were analysed using an LCmodel style analysis and quantified in absolute terms against total tissue water determined by biexponential analysis of the multi-echo water spectra. Metabolites (N-acetyl-aspartate, NAA, Creatine, Cre, Choline, Cho and *myo*-Inositol, *myo*-I) were also expressed as ratios relative to the Creatine peak. Statistical analysis was performed in SPSS 11.0 (SPSS Inc. Chicago, USA) using Mann-Whitney test and non-parametric correlation.

Results

A typical proton spectrum from the spinal cord voxel in an MS patient shown in figure 1.

Spinal Cord Metabolites: [NAA] was decreased by 25% in the spinal cord in the MS patients relative to control (table, p<0.05). [Cre] was decreased by 24% but this was not significant (p=0.11) (although the reduced NAA and an unchanged NAA/Cre ratio [1.86±0.56 patient vs 1.81±0.24 control)] suggests that [Cre] was decreased). The *myo*-I/Cre ratio was also significantly elevated in the cord of patients relative to control (1.39 ± 0.49 patient vs 0.90 ± 0.22 control, p=0.003).

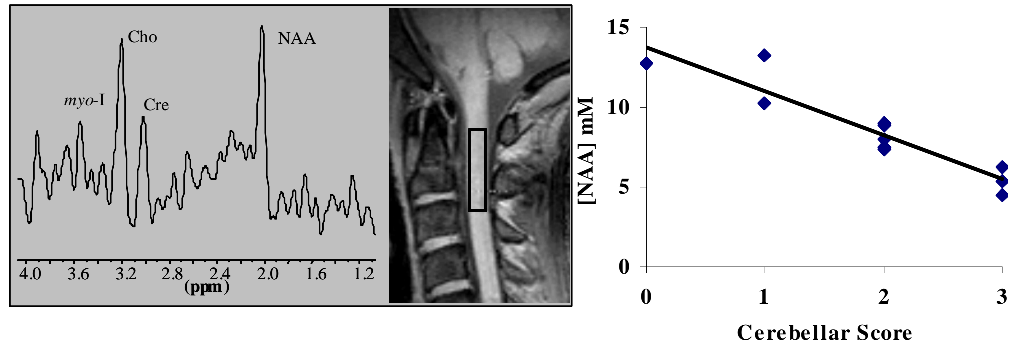
Brain Metabolites: No significant differences were observed in concentrations of NAA, Cre or Cho or peak ratios NAA/Cre, NAA/Cho, Cho/Cre between patients and controls. *Myo*-Inositol was significantly elevated (p<0.03) as was myoI/Cre (0.61 ± 0.12 patient vs 0.46 ± 0.13 control, p=0.02).

Imaging Changes: Brain lesion load varied considerably between patients (mean 11.7 ± 8.1 cm^3, range 0.4 to 21.6 cm^3). Cross sectional area of the spinal cord at the centre of the MRS voxel (C3) was significantly reduced in the MS patients compared to control (0.84±0.08 cm^2 vs 0.99±0.05 cm^2, p<0.001).

Correlations: Within the patient group, the concentration of NAA in the cord was significantly correlated with cerebellar score (figure 2, Spearmans rho -0.927, p<0.001) and showed a trend against the time taken to walk 10m (Spearmans rho -0.605, p=0.065). No correlations were found between cord metabolites (absolute or ratios), cord atrophy, EDSS or disease duration. Although mean [NAA] was not significantly reduced in brain compared to controls, values in individual subjects were significantly correlated with disease duration in the patients (Spearmans rho -0.74, p=0.009).

Discussion

In this cohort of patients with MS, [NAA] in the brain did not show significant differences relative to healthy controls , but was correlated with the duration of disease progression. In the spinal cord however, [NAA] was reduced significantly by 25%. Changes were not correlated with disease progression but were strongly related to clinical status. Previously reported increases in *myo*-Inositol were replicated here in brain and were also found in the spinal cord. These data are consistent with pathological loss of neurons observed in terminal disease[2,3]. The lack of coupling between brain and spinal cord [NAA] concentrations suggest that disease progression in the brain and cord is independent.



		[NAA] (mM)	[Cre] (mM)	[Cho] (mM)	[*myo*I] (mM)			[NAA] (mM)	[Cre] (mM)	[Cho] (mM)	[*myo*I] (mM)
Cord	Patient	**8.5 ± 2.8** **	4.9 ± 2.1	2.3 ± 0.8	6.3 ± 2.3	**Brain**	Patient	12.6 ± 1.8	8.9 ± 1.3	2.5 ± 0.3	**5.5 ± 1.4** **
	Control	11.3 ± 2.5	6.4 ± 2.1	2.5 ± 0.5	5.6 ± 1.8		Control	13.2 ± 1.7	8.9 ± 1.4	2.5 ± 0.3	4.0 ± 1.1

References

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