Elevated white matter myo-inositol in clinically isolated syndromes suggestive of multiple sclerosis


1NMR Research Unit and 2Epilepsy Research Unit, Institute of Neurology, 3National Hospital for Neurology and Neurosurgery, and 4Moorfields Eye Hospital, London, UK

Summary

Normal-appearing white matter (NAWM) in established multiple sclerosis has been shown to be abnormal using a variety of magnetic resonance (MR) techniques, including proton MR spectroscopy (1H-MRS), although the stage at which these changes first appear is less clear. Using a 1.5 T scanner and single-voxel 1H-MRS [TR 3000 ms, TE 30 ms, point-resolved spectroscopy (PRESS) localization], we determined NAWM metabolite concentrations in 96 patients a mean of 19 weeks (range 12–28 weeks) after onset of a clinically isolated syndrome (CIS) suggestive of multiple sclerosis and in 44 healthy control subjects. Absolute concentrations of N-acetyl-aspartate, total creatine and phosphocreatine (Cr), choline-containing compounds, glutamate plus glutamine, and myo-inositol (Ins) were estimated automatically using the LCModel. Compared with control subjects, the concentration of Ins was elevated in CIS NAWM (mean 3.31 mM, SD 0.86 versus mean 3.82 mM, SD 1.06; \( P = 0.001 \)). The increase in Ins was also seen in the patient subgroup with abnormal T2-weighted MRI (mean 3.88 mM, SD 1.10; \( P = 0.001 \)) and in those who satisfied the McDonald criteria for multiple sclerosis (mean 4.04 mM, SD 1.31; \( P = 0.001 \)). An increase in Cr was also observed in CIS NAWM (\( P = 0.023 \)), but other metabolites did not significantly differ between the whole CIS group and control subjects. There was no significant correlation between NAWM Ins and T2 lesion load. The early increase in Ins may reflect a process of pathogenic importance in multiple sclerosis NAWM. Follow-up studies will investigate whether the increase in NAWM Ins is of prognostic importance for future relapses and disability.

Keywords: MRS, NAWM; myo-inositol; multiple sclerosis; clinically isolated syndromes

Abbreviations: CIS = clinically isolated syndrome; Cho = choline; Cr = creatine and phosphocreatine; EDSS = Expanded Disability Status Scale; Glx = glutamate and glutamine; 1H-MRS = proton magnetic resonance spectroscopy; Ins = myo-inositol; MTR = magnetization transfer ratio; NAWM = normal appearing white matter; TE = echo time; TR = repetition time; tNAA = total N-acetyl-aspartate

Introduction

Multiple sclerosis is a common cause of chronic neurological disability in young adults. MRI readily identifies multifocal white matter lesions representing areas of demyelination, and is thereby useful in supporting the diagnosis of multiple sclerosis even after a single clinical episode (McDonald et al., 2001). It is also widely used to monitor disease progression both in natural history and therapeutic trial studies. However, conventional MRI does not detect the subtle histopathological changes that are described in the normal-appearing white matter (NAWM) in multiple sclerosis (Allen, 1979, 1981; Bitsch et al., 1999). In addition, the specificity of T2-weighted MRI is low: it does not differentiate between the distinct pathological processes involved in multiple sclerosis, such as inflammation, demyelination, remyelination, axonal loss and gliosis. These two factors are likely to contribute to the generally poor correlations observed between the total T2 lesion load and clinical disability in patients with multiple sclerosis.

Proton magnetic resonance spectroscopy (1H-MRS) is a non-invasive technique that enables the in vivo investigation of metabolic alterations associated with brain pathology, and provides a quantitative tool for investigating the abnor-
nalities in the NAWM (Urenjak et al., 1993; Husted et al., 1994; Bitsch et al., 1999). At longer echo times, N-acetyl-aspartate [with N-acetyl-aspartyl-glutamate designated tNAA; a metabolite almost exclusively found in neurons and their axonal projections (Simmons et al., 1991)], creatine and phosphocreatine (Cr), and choline-containing compounds (Cho) may be quantified, while at shorter echo times additional metabolite peaks may be seen from myo-inositol (Ins), a potential marker of glial cells (Brand et al., 1993; Bitsch et al., 1999) glutamate and glutamine (Glx), and mobile lipids (Wolinsky et al., 1990; Davie et al., 1994; Narayana et al., 1998).

1H-MRS studies in patients with established multiple sclerosis have demonstrated significant abnormalities in the concentration of metabolites in the NAWM, with notably reduced tNAA (Davie et al., 1995, 1997; Matthews et al., 1996; Rooney et al., 1997; Fu et al., 1998; Narayana et al., 1998; De Stefano et al., 1998, 1999, 2001; Leary et al., 1999; Sarchielli et al., 1999; van Walderveen et al., 1999, 2001; Suhy et al., 2000) and raised Ins (Kapeller et al., 2001, 2002; Chard et al., 2002b; Vrenken et al., 2003; Sastre-Garriga et al., 2003). A decrease in tNAA is thought to indicate axonal dysfunction or loss, while an increase in Ins has been proposed to reflect an increase in glial cell activity or numbers. The stage at which these abnormalities first appear is less clear since few studies have concentrated on NAWM in the earliest stages of multiple sclerosis, particularly within 1 year of clinical onset (Brex et al., 1999a; Tourbah et al., 1999). It is relevant to study this early phase of disease in order to search for prognostic markers for the future clinical course and to gain insights into early pathological and pathogenic processes.

In approximately 90% of multiple sclerosis patients, the first clinical manifestation is a clinically isolated syndrome (CIS). This can be defined as a solitary, inflammatory, demyelinating syndrome of acute onset in the CNS in an individual without a previous history of demyelination and in whom appropriate investigations have excluded alternative diagnoses. In a preliminary report of NAWM 1H-MRS in 20 patients with CIS, we found no significant change in the absolute concentrations of tNAA, Cho, Cr and Ins compared with controls (Brex et al., 1999a). However, the power of that study was substantially limited by the small size of the cohort studied. The aim of the present study was to investigate the NAWM more definitively by studying a much larger cohort of patients, over 90% of whom were studied within 6 months of presentation with a CIS. By investigating CIS subgroups with (i) MRI lesion abnormalities that are known to increase the likelihood of developing clinically definite multiple sclerosis (O’Riordan et al., 1998; Brex et al., 2002; Beck et al., 2003), and (ii) a diagnosis of multiple sclerosis already established using the new McDonald criteria, it was possible to infer the 1H-MRS findings in NAWM at the earliest clinically identifiable stage of multiple sclerosis.

Methods

Subjects

Patients

Patients who underwent 1H-MRS were participating in a prospective, multiparameter, MR and clinical follow up study of CIS patients first seen and investigated within 3 months of symptom onset. Full details of the study design are provided elsewhere (Brex et al., 1999b; Dalton et al., 2002). In all patients, appropriate investigations were undertaken to exclude alternative diagnoses. Disability was assessed using the Kurtzke Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). The study had approval from the National Hospital for Neurology and Neurosurgery ethics committee, and informed consent was obtained from all subjects before entry into the study. The 1H-MRS study, performed in 96 patients, took place at the first scheduled follow up visit 3 months after the baseline assessment; in more than 90% of cases this was within 6 months of onset of the CIS.

Controls

Using identical methods, 1H-MRS was also obtained from 44 healthy adult controls.

MRI protocol

All MR studies were performed with a 1.5 Tesla GE Signa Echospeed scanner (General Electric Medical Systems, Milwaukee, WI, USA). The patients had baseline T2-weighted and T1-weighted pre- and post-gadolinium (0.1 mmol/kg body weight) brain and spinal cord MRI within 12 weeks of their initial presentation (mean 5.9 weeks, SD 3.1, range 1–12 weeks). The acquisition parameters were as follows: (i) brain, 3 mm thick, contiguous, axial slices (256 × 256 matrix) with repetition time (TR) 3200 ms, echo time (TE) 15/90 ms for the T2-weighted sequences, and TR 600 ms, TE 17 ms for the T1-weighted sequence; (ii) spinal cord, 3 mm thick, contiguous, sagittal slices (256 × 256 matrix) with TR 2500 ms, TE 56/98 ms for the T2-weighted sequences and TR 500 ms, TE 19 ms for the T1-weighted sequence. They then underwent a repeat MRI of the brain approximately 3 months after the baseline scan (mean 13.1 weeks, SD 2.3, range 8–20 weeks after baseline scan, and mean 18.9 weeks, SD 3.5, range 12–28 weeks after the initial CIS). Baseline and 3-month follow-up brain scans were analysed for the presence and number of T2 and gadolinium-enhancing lesions by an experienced neuroradiologist blinded to the clinical data. After 3 months, the clinical and MRI findings were reviewed to determine whether patients fulfilled the McDonald diagnostic criteria for multiple sclerosis (McDonald et al., 2001). T2 brain lesion volume at baseline was measured from electronic images using a semi-automated contouring technique to outline lesions (Grimaud et al., 1996).

1H-MRS

1H-MRS was obtained at the same time as the 3-month follow-up MRI scan, a mean of 18.9 weeks (SD 3.5, range 12–28 weeks) after CIS onset in 96 patients. In 89 out of 96 patients (93%), the 1H-MRS study was performed within 6 months of the CIS onset. A fast spin echo (FSE) axial localizing scan (TR 3000 ms, TE 14/84 ms, matrix 256 × 192, slice thickness 5 mm, interslice gap 1.5 mm) was acquired first. Single voxel 1H-MRS was then acquired from the NAWM in the posterior parietal and centrum semi-ovale regions.
lesions consistent with demyelination. Twenty-seven (28%) patients fulfilled the McDonald criteria for multiple sclerosis at the time $^1$H-MRS was performed.

**NAWM Ins**
Compared with the control group, the concentration of Ins was significantly higher in the NAWM of the whole CIS group ($P = 0.001$), in those with abnormal T2-weighted MRI at baseline ($P = 0.001$), and in those with multiple sclerosis diagnosed using the McDonald criteria ($P = 0.001$) (Table 1, Fig. 1); it was also increased in those who did not satisfy the McDonald criteria ($P = 0.008$). No significant differences in NAWM Ins were observed between the patient subgroups, i.e. subjects with normal versus abnormal baseline MRI and those fulfilling the McDonald criteria for multiple sclerosis versus those who did not.

There were no significant correlations between the absolute concentration of Ins and the number and volume of T2 brain lesions at baseline ($r_x = 0.098, P = 0.342$ and $r_x = 0.097, P = 0.346$, respectively) or with EDSS at baseline ($r_x = -0.091, P = 0.379$).

**NAWM tNAA**
There was a trend for the CIS group to display lower tNAA than controls, more so in those with T2 lesions, but the difference was not significant (in CIS patients with T2 lesions versus controls, the decrease of mean tNAA was $-2.7\%, P = 0.104$; Table 1). No significant differences in NAWM tNAA were observed between the patient subgroups: those with a normal versus abnormal baseline MRI and those who satisfied the McDonald criteria for multiple sclerosis versus those who did not.

**Other NAWM metabolites**
There was an increase in Cr in the whole CIS group versus controls ($P = 0.023$; Table 1). This increase was also apparent in the subgroup that had abnormal T2-weighted MRI and in those with multiple sclerosis by the McDonald criteria. An increase of borderline significance was seen in Cho in those patients with multiple sclerosis by the McDonald criteria ($P = 0.040$; Table 1) and in Glx in those CIS patients with an abnormal T2 weighted scan ($P = 0.044$; Table 1). There were no significant differences in any of the metabolite concentrations between the patient subgroups, i.e. those with a normal versus abnormal baseline MRI and those who satisfied the McDonald criteria for multiple sclerosis versus those who did not. When patients scanned before 18 weeks were compared with those scanned after 18 weeks from the time of CIS, there was no difference in any of the metabolite concentrations (Ins, tNAA, Cho, Cr and Glx).
Fig. 1 (A) Axial T2-weighted MRI from a CIS patient, showing a voxel placed over an area of NAWM. (B) Spectrum from the NAWM of a CIS patient. (C) Spectrum from the NAWM of a control subject matched for age and gender.
Ratio measures using Cr as denominator

The ratio of NAWM Ins/Cr was significantly increased in CIS patients versus controls [mean (SD), 0.94 (0.27) versus 0.83 (0.21), \( P = 0.015 \)] but there was no significant difference in the Cho/Cr or Glx/Cr ratios. However, tNAA/Cr was significantly lower in patients versus controls [mean (SD), 0.94 (0.27) versus 0.83 (0.21), \( P = 0.001 \)].

Discussion

The main findings in this study were a significant increase in NAWM Ins and Cr concentrations in patients with CIS. The increase in Ins was more significant than that for Cr. Increased Ins was observed in the subgroup of CIS patients with abnormal scans, who are known from previous follow-up studies to have a high probability of developing clinically definite multiple sclerosis (Poser et al., 1983; Barkhof et al., 1997; Optic Neuritis Study Group, 1997; O’Riordan et al., 1998; Brex et al., 2002; Beck et al., 2003). NAWM Ins was also increased in those who had developed multiple sclerosis as defined by the McDonald criteria at the time of \(^1\)H-MRS, which was performed in over 90% of patients within 6 months of presentation with a CIS. Taken together, the results provide evidence that Ins is increased in NAWM in the earliest clinical stage of multiple sclerosis, although an increase was also observed in those CIS patients who did not satisfy the McDonald criteria.

In two previous studies of CIS NAWM, an increase of Ins was not reported (Brex et al., 1999a; Tourbah et al., 1999). One of these was a preliminary study that we performed in a small subgroup (\( n = 20 \)) of the present cohort, and the small sample size is likely to account for the negative result (Brex et al., 1999a). The other study was of a larger cohort (\( n = 42 \)), but measured metabolites as a ratio to Cr (Tourbah et al., 1999), rather than individual metabolite measures as used in the present study. The use of ratios is potentially less sensitive to disease effects in multiple sclerosis than the use of absolute values; measurement error may be increased when two measures rather than one are required and the use of the ratio to Cr also requires the assumption that Cr is normal in the patients’ NAWM. In this regard, it is noteworthy that there was evidence for a marginal elevation in Cr in the present CIS cohort. There have been other reports describing abnormalities of Cr in demyelinating lesions and multiple sclerosis NAWM (Rooney et al., 1997; van Walderveen et al., 1999; Brex et al., 1999a; Suhy et al., 2000; Vrenken et al., 2003), although this has not been a consistent finding. However, this suggests that Cr may not be a reliable internal standard when investigating the effects of demyelinating disease upon metabolite concentrations.

Because we only investigated one voxel, it is not possible to confirm whether an increase in Ins occurs more generally throughout the white matter. However, studies in cohorts with established multiple sclerosis have reported increases in NAWM Ins obtained from a whole cerebral slice that included frontal and parietal white matter (Kapeller et al., 2002; Chard et al., 2002b). Studies of multiple sclerosis NAWM using other quantitative MR techniques have also revealed widespread abnormalities (Ciccarelli et al., 2001; Griffin et al., 2002). It seems likely that the abnormality we observed is more widespread than just the single parieto-occipital white matter voxel that was studied. Another metabolite that occurs at the same point of the \(^1\)H spectrum as Ins is glycine. Glycine has a longer T2 than Ins and may be distinguished from the latter by obtaining a long TE spectrum. Although we did not perform a long TE acquisition, another study of CIS patients has done so and did not report an abnormal peak at this point in the spectrum (Tourbah et al., 1999). Furthermore, the concentration of glycine is approximately one-twentieth of that of Ins in normal white matter (Petroff et al., 1989). It is therefore likely that the observed increase is due to Ins rather than glycine. Short TE \(^1\)H spectra have contributions from broad macromolecular resonances which may overlap with the Ins.
resonance and it is possible that they may have contributed to the observed abnormality. We are confident that the increase in Ins was not due to inconsistency of water suppression because the automatic water suppression procedure was reliable throughout the study period and there is also evidence that metabolite measures obtained with the LCModel are insensitive to variations in water suppression (Provencher, 2001).

Several pathophysiological processes have potential to cause an increase in NAWM Ins in multiple sclerosis. Ins is preferentially concentrated in glial cells (Brand, 1993), although its function in the CNS is uncertain—one role may be to act as an osmolyte. An increase in the activity or number of glial and inflammatory cells has been reported in histopathological studies of multiple sclerosis NAWM. Specific findings include diffuse astrocyte hyperplasia and perivascular lymphocyte infiltrates (Allen and McKeown, 1979; Allen et al., 1981). Microglial activation is also described (Allen, 2001). A recent report, using a PET radioligand that identifies peripheral benzodiazepine receptors on activated microglia has indicated an increase in their activity in NAWM in multiple sclerosis (Debruyne et al., 2003). An increase in Ins greater than that seen in NAWM has also been reported in white matter lesions in multiple sclerosis (Davie et al., 1994), with a more marked increase occurring in T1 hypointense than isointense lesions (Brex et al., 2000). The higher level of Ins in lesions is consistent with pathological studies that reveal more extensive gliosis in lesions than NAWM.

We have previously reported an increase in Ins in the NAWM of three other cohorts of patients with demyelinating disease. In a group of patients with clinically definite relapsing remitting multiple sclerosis and disease duration less than 3 years, the increase in Ins was correlated with measures of functional impairment measured using the Multiple Sclerosis Functional Composite Scale (Chard et al., 2002b). In another cohort of patients who were followed up 14 years after presenting with a CIS, by which time most had developed clinically definite multiple sclerosis, the increase in Ins was correlated with disability measured using the EDSS (Kapeller et al., 2002). Most recently, we have observed an increased NAWM Ins in patients with primary progressive multiple sclerosis and again found that it was significantly correlated with disability (Sastre-Garriga et al., 2003). Taken together, these findings provide robust evidence that an increase in NAWM Ins is a consistent finding in multiple sclerosis and is of functional significance. Whether the early increase in Ins in our present study can be related to the future clinical course, in particular to the development of disability, is not known. Finding a reliable early prognostic marker for disability will assist with counselling individual patients and identifying those in whom early disease-modifying treatment is most needed. Presently available prognostic indicators, based on clinical features or conventional MRI lesion measures, are at best only modestly related to long-term disability (Weinshenker et al., 1989; Brex et al., 2002; Confavreux et al., 2003). Investigation of our cohort with long-term follow-up is being undertaken to determine whether Ins might contribute towards more reliable prediction.

The early increase in NAWM Ins was not correlated with T2 lesion load, and therefore may reflect, at least in part, an early aspect of the disease process that is independent of lesions. Given that MRI lesions are in general weakly related to disability and that other quantitative MR measures [e.g. magnetization transfer ratio (MTR), diffusion] of abnormality in normal-appearing brain tissues have revealed more robust correlations with clinical disease progression (Filippi et al., 1999, 2000, 2003a; Miller et al., 2003; Rovaris et al., 2003), the possibility arises that the pathological process underlying the early increase in NAWM Ins may have a pathogenic role in the disease. Previous studies have identified abnormalities in a number of MR measures in NAWM prior to the development of focal white matter lesions. These include the appearance of mobile lipid resonances on 1H-MRS (Narayana et al., 1998), reduced MTR (Filippi et al., 1998; Pike et al., 2000) and increased diffusion (Werring et al., 2000). Further studies are needed to clarify the pathological basis of the early Ins change and to follow the evolution of Ins abnormalities over time and their relationship to lesion evolution.

Although not significant, the mean tNAA in CIS patients was about 2.2% less than that found in healthy controls; as the reproducibility of metabolite measures for in vivo 1H-MRS studies is imperfect, our results cannot exclude the possibility of a small amount of early axonal damage or loss within the limits of measurement and biological variation. However, the reproducibility of tNAA is better than that of Ins (Chard et al., 2002a), suggesting that the magnitude of any early disease effect upon tNAA concentrations is markedly smaller than that upon Ins (mean Ins in CIS patients was about 15% higher than that found in controls).

Noting that methodological differences make direct comparisons between studies difficult, our finding of no significant reduction in tNAA is consistent with the previously reported normal tNAA/Cr ratio from NAWM in a group of patients with isolated optic neuritis compared with controls (Tourbah et al., 1999). It is, however, notable that in the present study the tNAA/Cr ratio was significantly reduced, evidently because Cr was increased in NAWM. As already discussed, these findings emphasize the value, where possible, of obtaining absolute metabolite concentration measures. Another recent study, in patients with clinically definite relapsing remitting multiple sclerosis and a disease duration less than 3 years, reported that, compared with controls, there was a significant reduction in multiple sclerosis NAWM tNAA (mean ~5%). This study used single-slice spectroscopic imaging through the cerebral hemispheres with the same LCModel analysis technique as in the present study (Chard et al., 2002b). Taking these findings together suggests that axonal loss or dysfunction in NAWM is minimal at multiple sclerosis onset with a CIS but becomes apparent
within a few years. Follow-up MRS of the present cohort is being undertaken to further investigate this hypothesis.

Our finding of no evident abnormality of NAWM tNAA contrasts with another recent study of CIS patients, which used a different acquisition method to quantify the concentration of tNAA from the whole brain and reported a 22.3% reduction in patients versus controls (Filippi et al., 2003b). The difference in findings may reflect the fact that the whole brain study included lesions and grey matter as well as white matter, or that the cohort studied was smaller and restricted to those with evidence fulfilling the McDonald criteria of dissemination in space using MRI alone or the combination of MRI and CSF findings. Another difference between the present approach and that for measuring whole-brain tNAA is that the former provides an automated and objective measurement, which is made from a narrow and well-defined resonance peak, whereas the latter produces a broad resonance that needs to be manually integrated. The whole brain analysis is therefore more subjective and has the potential to be less reproducible. Further studies that specifically explore disease effects upon grey matter and white matter metabolite profiles in patients with CIS are needed to clarify the whole brain tNAA observations.

We observed either marginal or no abnormalities in other metabolite concentrations in CIS NAWM. The borderline increases in Cho, Cr and Glx seen either in the whole CIS group or in some of the patient subgroups may be accounted for by an increase in cell turnover or metabolism, which might be expected to occur with inflammatory or glial reactive features such as have been described in multiple sclerosis NAWM. 1H-MRS-visible choline containing compounds are predominantly derived from cell membranes and may be elevated when there is increased cell turnover. An increase in total creatine and phosphocreatine may reflect greater cellular metabolic activity. Glx represents a combination of glutamate and glutamine, and an expansion in the numbers of glial cells might increase their total concentration. These findings, although in themselves non-specific and of marginal significance, support the view that the highly significant increase in Ins is likely to reflect similar pathological processes. The increase in Cr was seen in the group that had multiple sclerosis using the McDonald criteria, but not in those who did not. This suggests a potential for Cr to have a prognostic role; further follow-up is needed to address this issue.

Whilst considerable care was taken both when placing 1H-MRS voxels in NAWM and subsequently confirming their purity by reference to concurrently acquired structural images, it is possible that the results may have been influenced by the presence of small lesions. It was therefore reassuring to note that there was no clear association between T2 lesion loads and NAWM Ins concentrations, and this suggests that lesion contamination cannot account for the present observations.

In summary, this study highlights NAWM Ins as an in vivo measure that is of potential interest in studying multiple sclerosis. It is elevated at an early clinical stage within 6 months of onset with a CIS, and in the subgroup who can already be diagnosed as having multiple sclerosis at this time. Follow-up is now required to clarify the relationship between Ins abnormalities and the long-term clinical course.

Acknowledgements
We wish to thank the subjects who kindly agreed to take part in this study. We also wish to thank Daniel Altmann for statistical advice, Christopher Benton, Kelvin Hunter and Claire Middleditch for technical support, and Siobhan Leary, Peter Brex and Anand Trip for their support and advice. The NMR Research Unit is supported by The Multiple Sclerosis Society of Great Britain and Northern Ireland. K. T. M. F. is supported by Biogen and C. M. D. by Elan.

References


Elevated myo-inositol in the NAWM of CIS 1369