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Serial proton magnetic resonance spectroscopy in acute multiple sclerosis lesions

C. A. Davie, C. P. Hawkins, G. J. Barker, A. Brennan, P. S. Tofts, D. H. Miller and W. I. McDonald

NMR Research Group, Institute of Neurology, Queen Square, London, UK

Correspondence to: Dr C. A. Davie, NMR Research Group, Institute of Neurology, Queen Square House, London WC1N 3BG, UK

Summary

Serial proton magnetic resonance spectroscopy (MRS), was carried out at 1–2 monthly intervals on eight patients with multiple sclerosis who presented with evidence of a large acute cerebral white matter lesion. An MRS was obtained from acute lesions (volumes of interest 4–12 ml), which at presentation showed gadolinium-diethylenetriamine penta-acetic acid enhancement, and from similar volumes of normal appearing white matter lateral to the lesion and nearer the scalp. Follow-up ranged from 4 to 9 months (mean 6 months). Short echo spectra from acute enhancing lesions invariably showed the presence of large resonances at 0.9 and 1.3 p.p.m. compared with normal appearing white matter and healthy age matched controls, indicating that these peaks were not the result of voxel contamination from scalp fat. These resonances, which probably represent lipid products of myelin breakdown, were detected in lesions which had been enhancing for <1 month and remained elevated for a mean of 5 months (range 4–8 months). The results provide evidence that short echo proton MRS can detect myelin breakdown products and that myelin breakdown occurs during the initial inflammatory stage of lesion development. The ratio of N*-acetylaspartate (NAA) (a neuronal marker) relative to creatine was reduced in acute lesions and in normal appearing white matter. In six of the lesions studied there was, however, a subsequent rise in the NAA/creatine ratio indicating that axonal loss is not the only mechanism of reduction in the NAA/creatine ratio.*

Key words: NMR spectroscopy; multiple sclerosis; demyelination; lipids; *N*-acetylaspartate

Introduction

An important unresolved question about the pathogenesis of multiple sclerosis is when does demyelination occur? Though it has long been known that both inflammation and demyelination can be present in the same lesion (Frommann, 1867; Charcot, 1868; Dawson, 1916; Lumsden, 1970; Allen, 1991), the temporal pattern of evolution of each of these processes has not been elucidated by pathological studies which provide information only at a single time point. Serial MRI studies (Kermode *et al.*, 1990) have shown that the earliest detectable event in the development of a new lesion is a breakdown of the blood–brain barrier; there is experimental (Hawkins *et al.*, 1990) and post-mortem evidence (Katz *et al.*, 1990, 1993) that this indicates the presence of inflammation. However, conventional MRI has not yielded information about the time course of demyelination since it does not reveal normal myelin or myelin breakdown products.

Proton magnetic resonance spectroscopy (MRS) at long echo times (TEs) of 135 and 270 ms detects metabolites with long T_2 relaxation times, such as *N*-acetylaspartate (NAA), choline containing compounds, creatine/phosphocreatine and lactate. Proton MRS at short TEs of 10–30 ms detects additional metabolites with short T_2 relaxation times. These include mobile lipids, proteins, inositol, glucose and a number of the neurotransmitters such as glutamate and glutamine. The ability to detect mobile lipid resonances within multiple sclerosis lesions using proton MRS would provide, for the first time, a non-invasive tool with which to monitor myelin breakdown and to study the temporal relationship of inflammation and demyelination *in vivo*.

It has also been suggested that proton MRS may have a role in monitoring axonal loss (probably an important factor in the development of persistent disability in multiple sclerosis) since NAA, an amino acid clearly visible on proton MRS, is largely confined to neurons and their processes.

Recent technical advances in proton spectroscopy have improved its spatial resolution, allowing the study of volumes as small as 1 ml (Frahm *et al.*, 1990)—a common size for multiple sclerosis lesions. We have therefore carried out serial long and short TE proton MRS on patients with MRI or symptomatic evidence of acute cerebral lesions. Our aims were

(i) to determine whether myelin breakdown products are spectroscopically detectable at short TEs within the acute lesion; (ii) if they are, whether they are the result of voxel contamination from overlying scalp; (iii) to establish at what phase in the evolution of the lesion demyelination occurs; (iv) to elucidate the time course of changes in NAA.

Material and methods

Subjects

Eight patients (age range 20–48 years; mean age 28 years) were studied. Six patients had clinically definite multiple sclerosis and two had laboratory supported definite multiple sclerosis (Poser *et al.*, 1983). The disease severity ranged from mild to severe as measured on the expanded disability status scale (Kurtzke, 1983). Two patients had undergone treatment with intravenous methylprednisone 1 day prior to the first spectroscopic examination. Nevertheless, these lesions still enhanced after administration of gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA) (Magnevist, Schering) (Miller *et al.*, 1992). The patients were followed at 1–2 monthly intervals. Follow-up ranged from 4 to 9 months (mean 6 months). The patients were examined neurologically by one of the authors (C.A.D.) at entry into the study and at each follow-up session. The patients then underwent unenhanced MRI, localized proton MRS and finally Gd-DTPA enhanced MRI.

The study was approved by the Joint Ethics Committee at the Institute of Neurology and the National Hospital for Neurology and Neurosurgery, London. Informed consent was obtained from all patients prior to each study. The patients were judged to have an acute lesion (i) if there was an area of increased signal intensity within white matter on MRI which displayed Gd-DTPA enhancement and which had not been present on MRI carried out in the previous month (four patients who had undergone monthly unenhanced and enhanced brain scans over the previous year as part of other longitudinal studies) or (ii) if there had been an acute clinical relapse within the previous 2 weeks and there was an appropriately placed new lesion on MRI which enhanced after Gd-DTPA administration (four patients).

Eight healthy age-matched controls underwent MRI and localized proton MRS without administration of Gd-DTPA. A 17-year-old female with a lipoma in the quadrigeminal cistern was also studied.

Magnetic resonance imaging and spectroscopy

Both MRI and MRS were performed on a 1.5 T GE signa whole body scanner using a standard quadrature head coil. The study commenced with a T_2-weighted fast spin echo-imaging sequence [repetition time (TR) 3000 ms, fast TE (TE_f) 80 ms] (5 mm slices with 2.5 mm gap, 256×256 matrix, echo train length 8). After imaging, a volume of interest ranging between 3.5 and 12 ml was prescribed which in the patient group incorporated a new high signal lesion. Large lesions were chosen in order to minimize partial volume effects. An MRI of the voxel was then obtained to ensure accurate localization. Water suppressed ^1H spectra were obtained using a stimulated echo-acquisition mode (STEAM) sequence (Frahm *et al.*, 1987, 1989*a*). Acquisition parameters were TR 2000 ms, mixing time (TM) 12ms, TE 10 and 135 ms. The 256 averages were collected using an eight-step phase cycle in ~9 min. Points collected were 1024, with a spectral width of 750 Hz. Shimming to a line width of ~3 Hz and water suppression were reoptimized for each new location. In the control group, spectra were collected from an area of periventricular white matter.

In the patient group, long and short TE spectra were acquired from acute lesions on the first study and at all subsequent examinations. At the first study only, a separate location containing normal appearing white matter was also examined which was lateral to the lesion and closer to the scalp. After collection of the spectroscopic data, Gd-DTPA, 0.1 mM/kg was injected intravenously and T_1-weighted Gd-DTPA enhanced images were obtained (TR 500 ms, TE 40 ms).

Data processing included 1.5 Hz line broadening for filtering, Fourier transformation and zero order phase correction. No baseline correction was applied. Resonance areas were calculated by manual integration. Ratios for the various metabolites are expressed relative to creatine. The NAA and choline ratios were calculated from the spectra collected at 135 ms. Inositol ratios were calculated from spectra collected at 10 ms. Statistical analysis was performed using Student's *t* test. Values are expressed ±1 SD.

Results

Representative short echo and long echo spectra from a healthy 28-year-old female control are shown in Fig. 1A. As we have reported previously (Davie *et al.*, 1992) all spectra collected at a short TE from controls showed small resonances at 1.3 and 0.9 p.p.m. which have been assigned respectively to the methylene and methyl groups of lipid (May *et al.*, 1986). These assignments are corroborated by an experimental model, showing that signals appear at 0.9 and 1.3 p.p.m. in the proton spectrum after the induced formation of lipid droplets in myeloma cells (Callies *et al.*, 1993). *In vivo* confirmation of these assignments were obtained from the 17-year-old female patient with a lipoma situated in the quadrigeminal plate (Fig. 1B); large resonances were seen only at 0.9 and 1.3 p.p.m. (Fig. 1C).

A spectrum at a TE of 135 ms confirmed that there was no contribution from lactate in the resonance at 1.3 p.p.m. in healthy controls (Fig. 1A). Lipid resonances were not observed at this longer TE.

Resonances from the other metabolites have been assigned as follows (Frahm *et al.*, 1989*b*; Behar and Ogino, 1991): NAA at 2.02 and 2.6 p.p.m.; creatine/phosphocreatine at 3.04 p.p.m.; choline at 3.2 p.p.m.; myo-inositol at 3.54 p.p.m.; glutamate, glutamine and gamma aminobutyrate between 2.1 and 2.45 p.p.m.

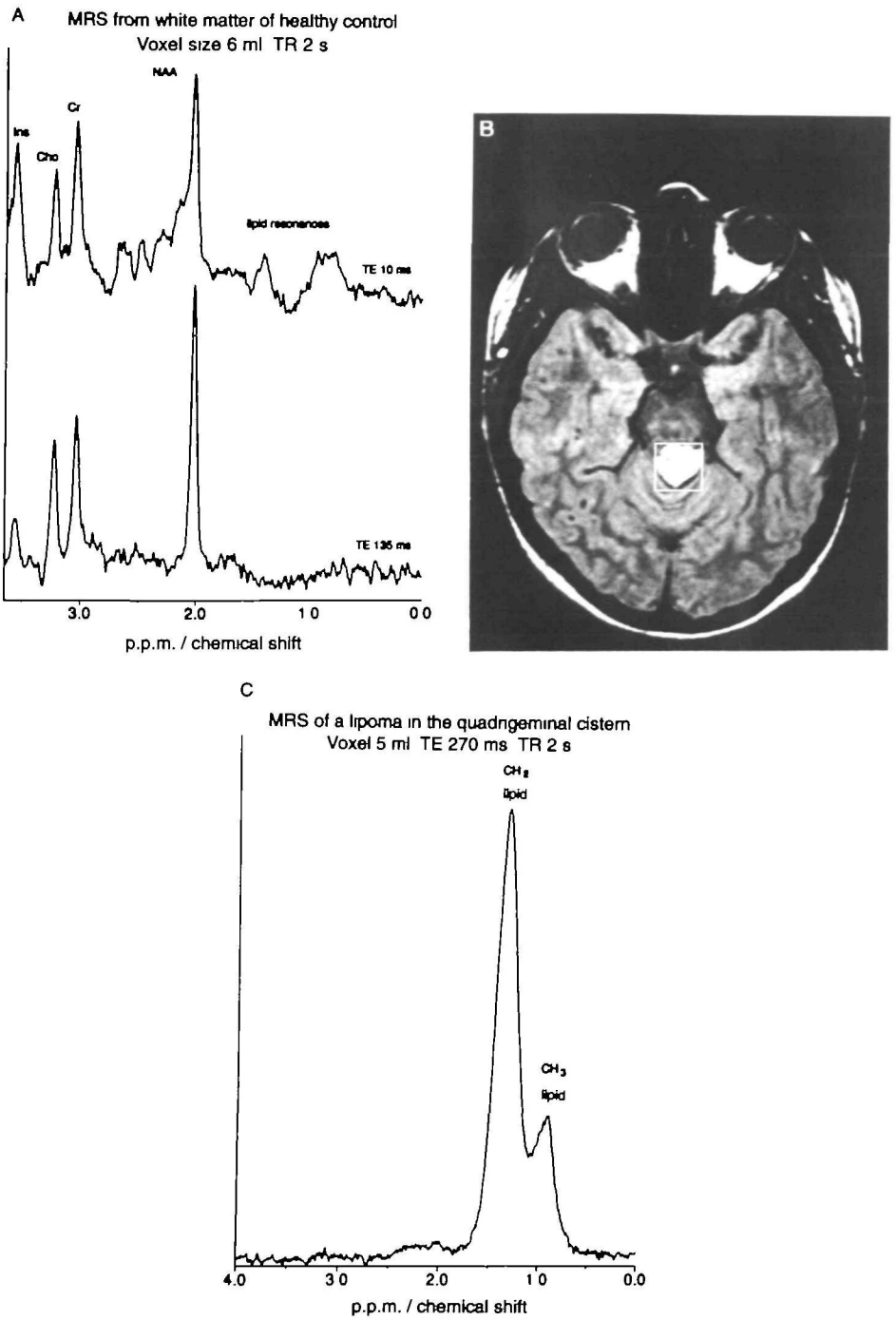

Fig. 1 A, proton spectra from a healthy 28-year-old female control taken from periventricular white matter. The upper spectrum is at TE of 10 ms. The lower spectrum is at a TE of 135 ms. Cho = choline; Cr = creatine; Ins = inositol; NAA = *N*-acetylaspartate. B, C, T_2-weighted NMR scan (TR 3000 ms, TE_f 80 ms) and short echo spectrum (TR 2 s, TE 10 ms) from a 17-year-old female with a lipoma in the quadrigeminal cistern. The spectrum taken from a voxel centred on the lipoma shows resonances from the methyl lipid group at 0.9 p.p.m. and from the methylene group at 1.3 p.p.m.

Short echo time spectra: patients

Short echo spectra in the patient group showed large resonances at 0.9 and 1.3 p.p.m. from Gd-DTPA enhancing lesions (Fig. 2), when compared with the small resonances seen in the regions of normal appearing white matter lateral to the lesion and in white matter from healthy controls. In one 36-year-old female with clinically definite multiple sclerosis it was possible to study a number of volumes from the lesion and surrounding

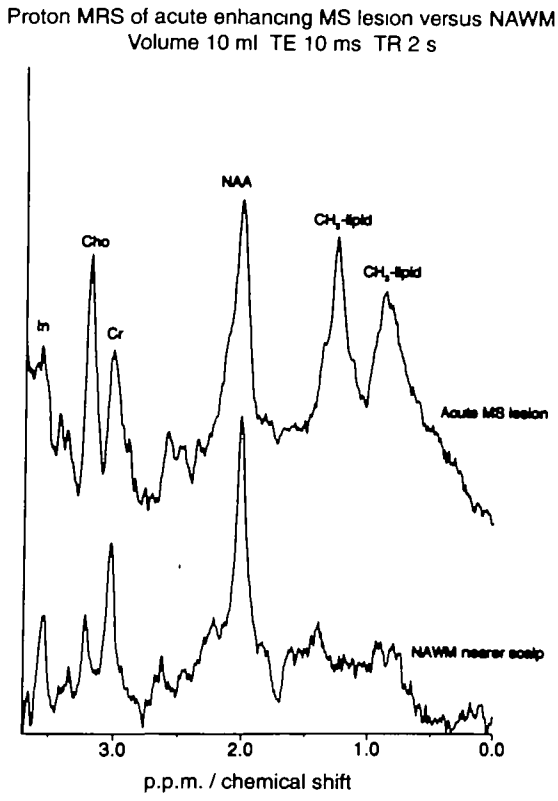

Fig. 2 Short echo time spectra (TR 2 s, TE 10 ms) from a 48-year-old female with clinically definite, relapsing–remitting multiple sclerosis. The upper spectrum is from a large gadolinium enhancing lesion which had been clinically symptomatic for 10 days. The lower spectrum is from an area of normal appearing white matter lateral to the lesion and nearer the scalp. NAWM = normal appearing white matter; NAA = *N*-acetylaspartate; Cho = choline; Cr = creatine, Ins = inositol. (Reproduced by kind permission of *The Lancet*.)

normal appearing white matter. A spectrum (Fig. 3, spectrum A) was collected from a 12 ml volume which included most of a region of high signal as shown on a T_2-weighted scan (Fig. 3, image A). When the voxel was reduced to a volume of 4 ml and taken from the centre of the lesion (Fig. 3, image B), the changes were far more striking (Fig. 3, spectrum B); this region was subsequently shown to enhance (Fig. 3, image D). A spectrum obtained from a 4 ml volume incorporating part of the lesion lateral to the region of enhancement and including normal appearing white matter (Fig. 3, spectrum C and image C) showed only small lipid resonances, consistent with the evidence that these non-enhancing areas surrounding enhancing active lesions are the result of oedema (Larsson *et al.*, 1988; McDonald *et al.*, 1992). In addition, the small size of these lipid signals (which are at control levels) from a volume nearer the scalp makes it highly unlikely that the large resonances from the centre of the lesion are due to contamination from scalp fat.

In seven patients, these abnormal lipid signals gradually resolved to control levels in from 4 to 8 months (mean ~5 months) (Fig. 4), whereas Gd-DTPA enhancement had ceased in all patients by 2 months. One patient followed to date, for only four months, still has increased lipid resonances.

In three of the patients there was the suggestion of a separate resonance seen at 1.4 p.p.m. (Fig. 5). This resonance was only visible at relatively short TEs reflecting the short T_2 of its components.

Inositol/creatine ratios were elevated within acute enhancing lesions (0.95 ± 0.16) compared with areas of normal appearing white matter (0.7 ± 0.1, $P < 0.01$) and white matter from healthy age matched controls (0.62 ± 0.2, $P < 0.003$). The inositol/creatine ratio in the lesion group remained elevated throughout follow-up. There was no significant difference between inositol/creatine ratios in the control group and normal appearing white matter ($P < 0.2$).

Long echo time spectra: patients

There was a reduction of NAA/creatine ratios in acute enhancing lesions (1.35 ± 0.27, $P = 0.001$) and normal appearing white matter (1.6 ± 0.22, $P < 0.02$), compared with controls (1.84 ± 0.2). In five of these lesions there was a subsequent further reduction in NAA/creatine ratios (mean 1.16 ± 0.15) with maximum reduction seen between 1 and 4 months (mean 2.2 months). In six of the lesions, there was a later rise in the NAA/creatine ratio which began between 4 and 6 months (Fig. 6). The mean NAA/creatine ratio at presentation in these six lesions was 1.41 ± 0.27 falling to 1.1 ± 0.15 ($P = 0.006$) between 1 and 4 months and rising to 1.57 ± 0.22 ($P = 0.001$) between 4 and 8 months. The mean NAA/creatine ratio in all eight lesions over time is shown in Fig. 7.

The mean choline/creatine ratio in the lesions when first studied was elevated (1.26 ± 0.26). This was significantly greater than in normal appearing white matter, (0.82 ± 0.13, $P = 0.006$) and controls (0.92 ± 0.08, $P = 0.01$). With follow-up the choline/creatine ratio returned to control levels in five out of eight lesions over 2–6 months. Although the mean choline/creatine ratio was lower in normal appearing white matter compared with controls, this was not statistically significant ($P < 0.09$).

Two of the lesions showed evidence of lactate production. In one, lactate was visible at the first study, 3 weeks after symptom onset. Spectroscopy 1 month later, after Gd-DTPA enhancement had ceased, showed resolution of the lactate peak. In the second patient, spectra acquired from a large enhancing lesion 10 days after symptom onset showed no lactate signal. However, lactate was visible 1 month later when Gd-DTPA enhancement had ceased. The lactate signal was no longer visible on spectroscopy 10 weeks after symptom onset.

Discussion

The outstanding findings in this study are the regular detection of small lipid resonances in normal white matter using short echo time proton spectroscopy, the marked increase in lipid resonances in association with the inflammatory phase of the new multiple scerosis lesion and the partially reversible reduction in the NAA/creatine ratio in new multiple sclerosis lesions.

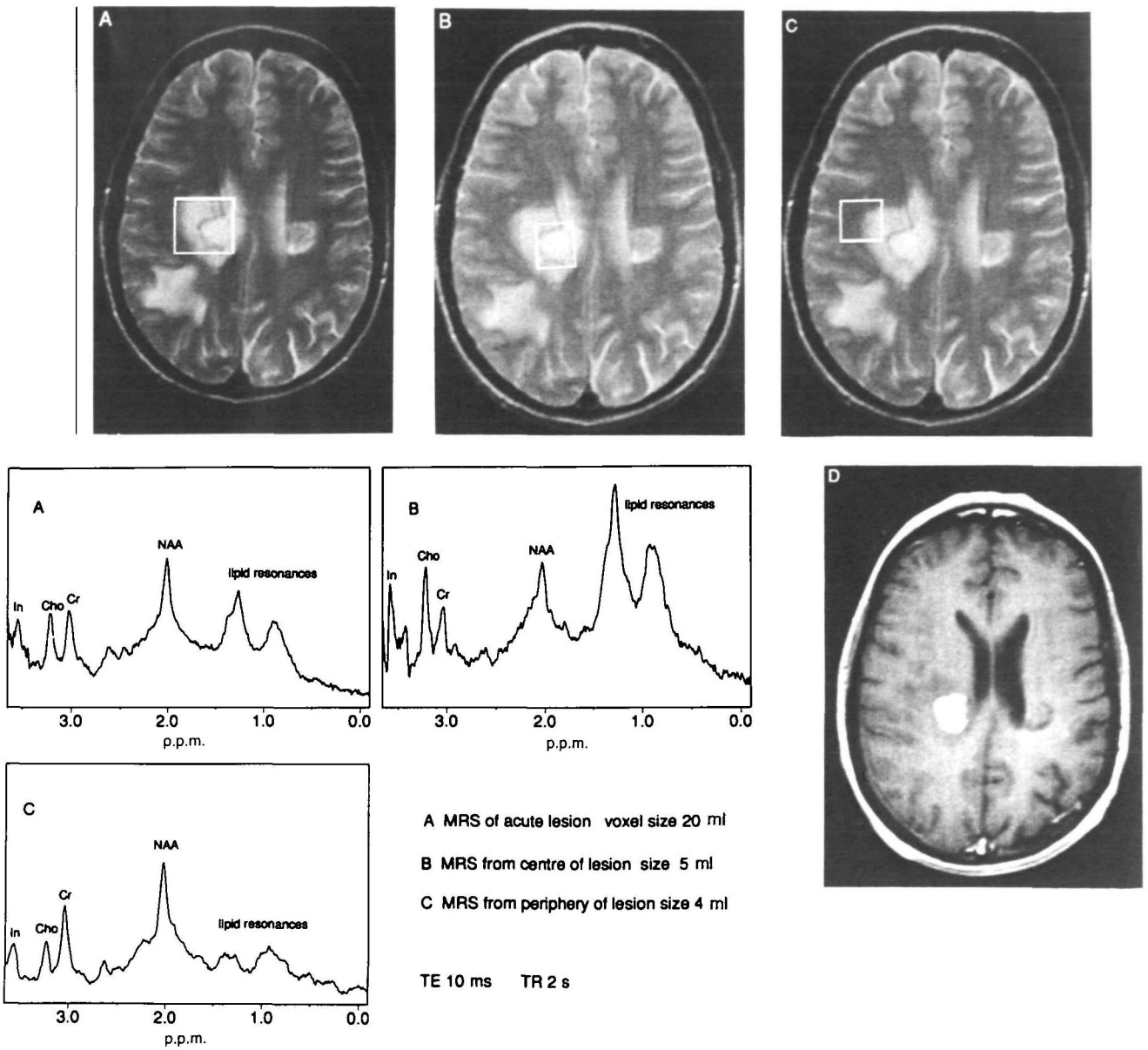

Fig. 3 Images A, B, C and D with spectra A, B and C. Magnetic resonance images and spectra from a 36-year-old female with clinically definite, secondary progressive, multiple sclerosis. Images A, B and C are T_2-weighted scans (TR 3000 ms, TE_f 80 ms) which show the respective volumes of interest from which spectra A, B and C were obtained (*see* text). Image D is a T_1-weighted scan (TR 500 ms, TE 40 ms) showing the area of Gd-DTPA enhancement within the lesion from which spectrum B was obtained. The increased lipid resonances are maximal in the area of Gd-DTPA enhancement.

Increased lipid resonances and the course of demyelination

We have shown that small resonances at 0.9 and 1.3 p.p.m. are a consistent finding in normal white matter of healthy individuals and multiple sclerosis patients. However, in large acute multiple sclerosis lesions, there was a marked increase in these resonances which have been assigned to the methyl and methylene groups of lipid, respectively (Callies *et al.*, 1993).

Some investigators (Wolinsky *et al.*, 1990; Larsson *et al.*, 1991; Davie *et al.*, 1993; Koopmans *et al.*, 1993) have observed increased resonances from multiple sclerosis lesions which have been attributed to mobile lipid, while others have not (Bruhn *et al.*, 1992; Grossman *et al.*, 1992; Poser *et al.*, 1992). When lipid peaks have been described, no consistent relationship has hitherto emerged, either with the apparent age of the lesion or the presence of Gd-DTPA enhancement (Narayana *et al.*, 1992). Systematic serial observations have not, however, been performed. Moreover, doubts have arisen as to whether the lipid peaks described have been due to extra-voxel contamination from the scalp. In the present study, two observations strongly suggest that these elevated peaks are not the result of scalp

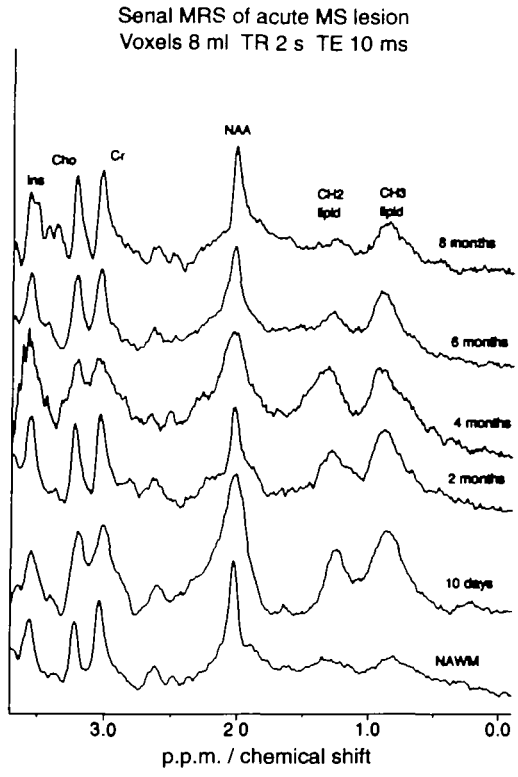

Fig. 4 Serial short echo time spectra (TR 2 s, TE 10 ms) at 2 monthly intervals taken from an acute lesion showing gradual resolution of lipid resonances over time. The lower spectrum is from an area of normal appearing white matter (NAWM) lateral to the lesion collected at the time of the first study – 10 days after symptom onset.

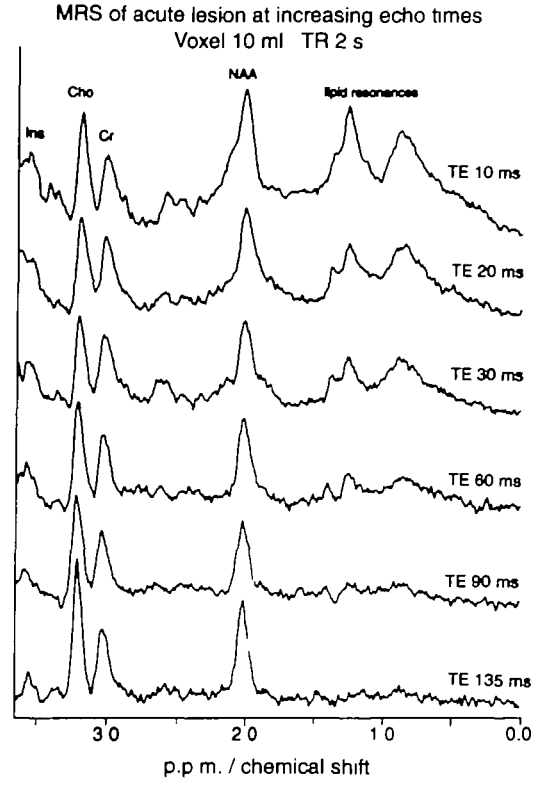

Fig. 5 Proton spectra from the same lesion as shown in Fig. 2. These spectra were obtained at progressively increasing TEs from 10 to 135 ms and show the resultant decrease in lipid signal at 0.9 and 1.3 p.p.m. as the TE increases.

contamination: first, they were consistently and markedly higher in the acute lesions than from an area of normal appearing white matter closer to the scalp; secondly, they consistently decreased towards normal levels over several months.

There are a number of possible reasons why previous studies have not revealed increased lipid resonances in multiple sclerosis lesions. First, the lesions studied were often small and the voxels could have included a considerable proportion of normal appearing white matter resulting in partial volume effects. In the present study, we concentrated on large lesions in order to avoid this problem. Secondly, a number of the non-enhancing lesions may have been chronic and the present study shows that abnormal lipid peaks will have disappeared in most lesions older than 6 months. Thirdly, a number of published studies have used TEs of 20–30 ms. Lipid has a very short T_2 (Fig. 5) and the increased signal may therefore not be as obvious at these slightly longer TEs, especially if the lesions are several months old.

In the present study, increased lipid resonances were seen in all lesions which displayed Gd-DTPA enhancement. Indeed, when lesion size allowed more precise resolution, this increase was maximal in areas of enhancement (Fig. 3, spectrum B). Four lesions had not been apparent with or without enhancement on scans in the previous 4 weeks. Since in multiple sclerosis, enhancement indicates an increase in permeability of the blood–brain barrier in association with inflammation (Katz *et al.*, 1990, 1993; Kermode *et al.*, 1990) we infer that demyelination occurs during the inflammatory phase of lesion development. Just how early in this phase it occurs, or whether it might precede it, is unknown, though recent observations on acute optic neuritis have shown that delayed evoked potentials (indicating the presence of demyelination) can be seen in enhancing lesions within 48 h of the onset of symptoms (S. Jones, R. Kapoor and W. I. McDonald, unpublished observations). Since enhancement is the earliest detectable event in the evolution of a new lesion and may precede the onset of symptoms (Barratt *et al.*, 1988), we conclude that demyelination occurs early in its development.

Increased lipid peaks were detectable in new lesions for 4–8 months. Histopathological studies of demyelination (Adams *et al.*, 1989) have shown lipid, in the form of triglyceride and cholesterol ester, in lesions which have been symptomatic for up to 26 weeks. Similar observations in cerebral infarction (Stehbens, 1972) show lipid laden macrophages which are detectable for a minimum of 3 months and in larger lesions for several years. The time course of increased lipid signal detected by proton MRS is thus compatible with the histologically determined time course of disappearance of lipid laden macrophages from areas of acute myelin destruction. Such destruction is, of course, not peculiar to multiple sclerosis and

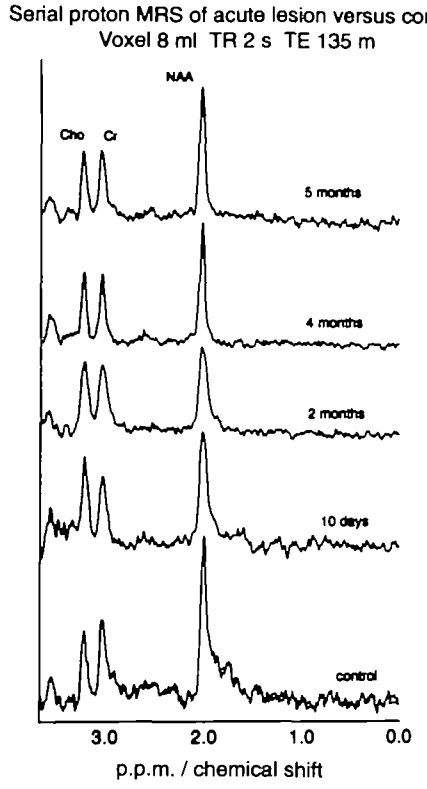

Fig. 6 Serial long echo spectra (TR 2 s, TE 135 ms) taken at 1–2 monthly intervals from an acute lesion showing a fall in the NAA signal relative to creatine and then a subsequent rise over time. The lower spectrum is from a healthy age-matched control.

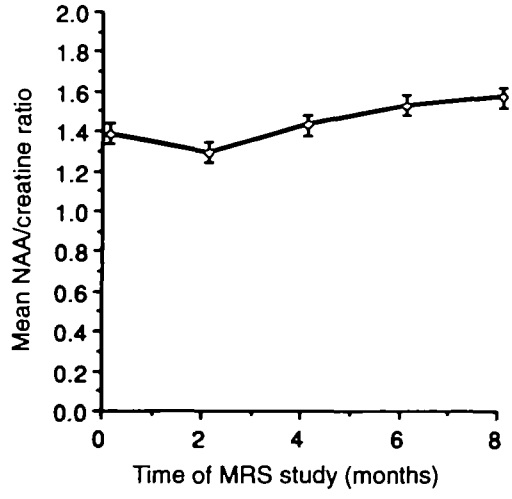

Fig. 7 Mean NAA relative to creatine ratio of the eight acute lesions studied over time. Error bars indicate standard error.

it is likely that abnormal lipid resonances will be found in a wide range of pathological conditions, including vascular and granulomatous disorders. Whether a pattern of spectral changes will emerge which is characteristic of multiple sclerosis remains to be seen.

A number of the acute lesions studied showed a resonance at 1.4 p.p.m. This resonance was more prominent at TEs of 20 and 30 ms (Fig. 5), indicating that it has a longer T_2 than the methylene group at 1.3 p.p.m. It is at present uncertain which metabolite this signal is due to, though the polypeptide thymosin $\beta 4$, found in macrophages and in a subset of oligodendrocytes, produces resonances at this region of the spectrum (Kauppinen *et al.*, 1992). There is some preliminary evidence that other macromolecules, e.g. myelin basic protein, may also produce resonances in this part of the spectrum (S. Davies, unpublished observations).

Reversible reduction in NAA/creatine ratio

Previous studies have reported a reduction in NAA/creatine ratios both in acute (Miller *et al.*, 1991; Arnold *et al.*, 1992; Bruhn *et al.*, 1992) and chronic lesions (Matthews *et al.*, 1991; Van Hecke *et al.*, 1991) in multiple sclerosis. The function of NAA is unknown. It is found almost exclusively in neurons and their processes in the adult brain (Birken and Oldendorf, 1989; Simmons *et al.*, 1991) though it has also been found in the oligodendrocyte type 2 astrocyte progenitor cells (Unrejak *et al.*, 1993). It has been suggested that the reduced NAA/creatine ratio in multiple sclerosis lesions is due to axonal loss (Bruhn *et al.*, 1992) which in chronic multiple sclerosis lesions may be extensive (Adams *et al.*, 1989) though it is only exceptionally so in acute lesions. In this study we observed a reduction in NAA/creatine ratios in acute lesions (Arnold, 1992) which was partially reversible over 4–8 months. A simple loss of axons cannot account for these reversible changes. A number of factors may be involved. First, alterations in the chemical environment of the lesion could alter the relaxation times of NAA, thus affecting signal intensity. However, this is unlikely. If one assumes a T_1 value of 1500 ms for normal cerebral white matter (Frahm *et al.*, 1989*b*), it would require a 75% increase in the T_1 of a lesion to explain the reduction in NAA signal we have observed. Such a change is improbable and spectroscopy of animals with acute experimental allergic encephalomyelitis has shown no changes in the T_1 or T_2 values of NAA (Inglis *et al.*, 1992).

Secondly, we and others have used creatine/phosphocreatine as an internal standard of reference, since these two compounds are in chemical equilibrium and their total concentration should accordingly remain constant. Frahm *et al.* (1989*b*) have calculated the cerebral concentration of total creatine as between 10 and 11 mM. If there were a reduction in the total creatine concentration which continued to fall after stabilization of NAA levels, the NAA/creatine ratio would rise. This hypothesis is not, however, in keeping with a study using phosphorus MRS in which there was an increase in phosphocreatine levels in multiple sclerosis lesions compared with healthy controls (Minderhoud *et al.*, 1992).

Thirdly, oedema is a prominent feature of the acute multiple sclerosis lesion (McDonald *et al.*, 1992) and it is possible that the relative number of axons per unit volume in the white matter affected by the lesion is at first reduced and later increases as the oedema is absorbed. Another possibility which is testable

experimentally, is that the function of the mitochondria (where NAA is formed) (Patel and Clark, 1979) is reversibly impaired during inflammation. Finally, there is the possibility that adult oligodendrocyte type two astrocyte progenitor cells containing NAA proliferate locally or migrate into the lesion in order to promote remyelination. Though the latter is extensive in some lesions (Prineas *et al.*, 1993), it is not in others, and whether the magnitude of the change in NAA can be accounted for in this way is unknown.

Other observations

The increase in choline/creatine has previously been interpreted as evidence for demyelination because of the abundance of choline-containing compounds in myelin. They are, however, abundant in all cell membranes (McIlwain and Bachelard, 1985) and as the study of acute experimental allergic encephalomyelitis (in which there is inflammation, but no demyelination) shows (Brenner *et al.*, 1993), a large increase in the choline/creatine ratio can be produced by the increased membrane turnover associated with inflammatory cellular infiltration. This is likely to contribute to the changes in the choline/creatine ratio we and others have observed (Matthews *et al.*, 1991).

The elevation in the inositol/creatine ratio in the lesion group is in agreement with previous studies (Bruhn *et al.*, 1992; Koopmans *et al.*, 1993). The function of inositol (an osmolyte) is uncertain and it is not yet clear why the inositol/creatine ratio is increased in multiple sclerosis.

Implications for monitoring therapy

The reliable detection of lipid breakdown by spectroscopy has implications for monitoring treatment in multiple sclerosis. β-Interferon reduces the frequency of evidence of new disease activity as judged by unenhanced MRI (IFNB Multiple Sclerosis Study Group, 1993: Paty *et al.*, 1993), but its mechanism of action is uncertain. It will be important to determine whether it (and future putative therapeutic agents) have differential effects on inflammation and demyelination. The former can be assessed by Gd-DTPA enhanced MRI, and the latter now by short echo time proton MRS. The observation of abnormalities in normal appearing white matter is probably due to the presence of microscopic demyelination (Allen, 1991) and is thus a potential measure of the extent of the disease process and possibly a prognostic indicator. Accordingly, it will be important to monitor not only the visible lesions, but the apparently uninvolved white matter as well.

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