In vivo evidence for axonal dysfunction remote from focal cerebral demyelination of the type seen in multiple sclerosis

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Summary
To test for axonal damage or dysfunction in white matter tracts remote from acute demyelinating lesions, we used brain proton magnetic resonance spectroscopic imaging to measure changes in N-acetyl aspartate (NAA), an index of neuronal integrity, in the white matter of the normal-appearing hemisphere of three patients with large, solitary brain demyelinating lesions of the type seen early in multiple sclerosis. During the acute phase of their disease, all patients showed normal ratios of NAA to creatine (Cr) resonance intensity throughout the hemisphere contralateral to the lesion. However, on examination 1 month later, all of the patients showed abnormally low NAA/Cr resonance intensity ratios (reduction of NAA/Cr by 22–35%) in voxels of the contralateral hemisphere which were homologous to the demyelinating lesion. Other voxels in the normal-appearing hemisphere showed normal NAA relative resonance intensities. The decrease in NAA/Cr in voxels of the normal-appearing hemispheres resolved in all patients after 6 months, with a time course similar to that observed for NAA from voxels within the lesions. We conclude that effects of damage or dysfunction to axons traversing inflammatory lesions can be transmitted over long distances in the normal-appearing white matter. Such remote, secondary effects may be an expression of dysfunction of axons in projection pathways or of the reorganization of functional pathways seen in brains recovering from an acute injury.

Keywords: demyelination; axon; magnetic resonance spectroscopy; multiple sclerosis; N-acetyl aspartate

Abbreviations: Cho = choline; Cr = creatine; NAA = N-acetyl aspartate; MR = magnetic resonance; MRSI = magnetic resonance spectroscopic imaging

Introduction
Proton magnetic resonance (MR) spectroscopic imaging (MRSI) is being used in an increasing number of clinical studies to supplement conventional diagnostic neuroimaging with spatially localized biochemical information (Arnold and Matthews, 1996). This MR technique allows the non-invasive quantitative monitoring of brain N-acetyl groups, which are present prevalently in N-acetyl aspartate (NAA), a metabolite localized almost exclusively in neurons and neuronal processes in the mature brain (Simmons et al., 1991; Moffett et al., 1991). The resonance intensity of NAA therefore provides an index of neuronal integrity (Birken and Oldendorf, 1989; De Stefano et al., 1995b).

Large decreases in brain NAA were observed in the earliest single-voxel proton MR spectroscopy studies of patients with multiple sclerosis (Arnold et al., 1990; Wolinsky et al., 1990). Subsequent studies have confirmed this finding and have suggested that the extent of axonal damage is a major determinant of disability (Matthews et al., 1991; Miller et al., 1991; Grossman et al., 1992; Davie et al., 1994). Chronic decreases in brain NAA probably reflect neuronal or axonal loss, which may be directly responsible for the chronic disability that accumulates in this disease (Davie et al., 1995; De Stefano et al., 1998; Matthews et al., 1998). Reversible decreases in NAA can be explained by reversible neuronal or axonal metabolic dysfunction and/or volume loss (De Stefano et al., 1995b; Matthews et al., 1995).
The observed decreases in NAA in multiple sclerosis patients are not restricted to lesions, but are present also in white matter that appears normal on conventional MRI (Davies et al., 1995; Narayanan et al., 1997; Fu et al., 1998). Evidence of axonal damage in the normal-appearing white matter of multiple sclerosis patients can also be found in neuropathological studies (Ferguson et al., 1997; Trapp et al., 1998). Since axons traversing lesions project through normal-appearing brain, abnormalities of NAA in normal-appearing white matter may reflect axonal damage occurring in lesions. However, axonal damage and related decreases in NAA in the normal-appearing white matter of patients with multiple sclerosis could also be a consequence of a more generalized process due either to multifocal pathology not associated with visible, macroscopic lesions or to the effects of diffusible substances that impair neuronal functions (Lumsden, 1970; Moudjian et al., 1991; Moreau et al., 1996; Koller et al., 1996; Pike et al., 1998).

In order to test for axonal damage or dysfunction well outside demyelinating lesions, we examined serial proton MRSI data of three patients who presented with a large, solitary, demyelinating brain lesion. This allowed us to assess the temporal evolution of the biochemical pathology occurring in normal-appearing brain remote from a new focal, demyelinating lesion.

Methods

Patient population

We studied three female patients (aged 19, 26 and 27 years) who were admitted to the Montreal Neurological Hospital because of motor and sensory impairment on the right side of the body that had evolved over the preceding days [Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) = 7.5, 7 and 8, respectively]. Details of their clinical histories are reported elsewhere (De Stefano et al., 1995a). In all patients, a conventional MRI performed a few days after the onset of symptoms showed a large lesion localized in the left parietal white matter and adjacent to the corpus callosum. A stereotaxic needle biopsy was performed in the three patients to differentiate between idiopathic demyelination, neoplasm and infection. In all subjects, the biopsies revealed a prominent lymphocytic cuffing of vessels, scattered reactive astrocytes, numerous foamy macrophages and a reduced number of myelin sheaths. All features were consistent with a primary demyelinating lesion of the type seen in multiple sclerosis (Zagzag et al., 1993). Silver preparations or other stains for the identification of axons were not performed. All patients were treated with high doses of corticosteroids, and all improved clinically (EDSS 1 month after the acute episode = 5, 4 and 6 in patients 1, 2 and 3, respectively). No other acute attacks occurred during 6 months of follow-up (EDSS 6 months after the acute episode = 3, 4 and 2 in patients 1, 2 and 3, respectively). Subsequently (~7 years later), one of the three patients had a clinically relevant episode of demyelination and therefore met the criteria for clinically definite multiple sclerosis. Such a long clinical follow-up was not available for the other two patients. Patients and normal subjects gave informed consent to participation in the study, which was approved by the Research Ethics Committee of the Montreal Neurological Institute and Hospital.

Proton MRI/MRSI of the brain

Combined proton MRI and MRSI examinations of the brain were obtained in a single session for each examination using a Philips Gyroscan operating at 1.5 T (Philips Medical Systems, Best, The Netherlands). For each patient, an initial proton MRI/MRSI examination was performed within 1 week of the onset of symptoms. Patients underwent subsequent MRSI examinations ~1 month and 6 months later. Multislice images obtained in the coronal and transverse planes [TE (echo time) = 2100 ms; TR (repetition time) = 5000 ms; field of view, 250 × 250 mm; slice thickness 6 mm] were used to select an intracranial volume of interest for spectroscopy. A volume of interest measuring ~80 mm anteroposteriorly × 20 mm craniocaudally × 90 mm left–right was positioned to include the lesion and the homologous region of the contralateral hemisphere. This was kept constant in size and location in subsequent examinations. Two-dimensional spectroscopic images were obtained using a 90°–180°–180° pulse sequence (TR = 2000 ms, TE = 272 ms, 250 × 250 mm field of view, 32 × 32 phase-encoding steps, one signal average per step) as previously described (De Stefano et al., 1995b). Magnetic field homogeneity was optimized to a line width of ~5 Hz over the volume of interest using the proton signal from water. Water suppression was achieved by selective inversion of the water resonance prior to volume selection using an adiabatic inversion pulse and adjustment of the waiting time so that the spectrum was acquired when the water signal passed through zero (Luyten et al., 1989). Prior to the water-suppressed acquisition, another MRSI was acquired without water suppression (TR = 850 ms, TE = 272 ms, 250 × 250 mm field of view, 16 × 16 phase-encoding steps) to allow B0 homogeneity correction.

Proton MRSI data analysis

Post-processing of the raw data was done on a SUN/SPARC system using SUNspec1 software (Philips Medical Systems, Best, The Netherlands) as previously described (De Stefano et al., 1995b). The non-water-suppressed MRSIs were zero-filled to 32 × 32. A mild Gaussian k-space filter and an inverse 2D Fourier transformation was then applied to both the water-suppressed and the unsuppressed MRSI. Artefacts present in the time-domain water-suppressed signal due to static magnetic field inhomogeneities and time-varying gradients were corrected by dividing the water-suppressed MRSI signal by the non-water-suppressed signal (den Hollander et al., 1991), a procedure which does not affect
Fig. 1 Proton brain MRI/MRSI examinations of patient 1 performed during the acute phase of a demyelinating disease (left), 1 month later (centre) and 6 months later (right). Conventional proton MRI examinations show a large, solitary, demyelinating lesion that decreases in size over time (top panels). Volumes of interest for spectroscopy are shown by the dotted line in each transverse MRI. Averaged spectra from voxels located in normal-appearing white matter contralateral and homologous to the demyelinating lesion (small squares in top panels) are shown in the bottom panels. Note the significant decrease in the NAA/Cr resonance intensity 1 month after the acute phase of the disease (bottom centre) and its complete recovery by 6 months (bottom right).

Table 1 Values of NAA/Cr in brain voxels contralateral and homologous to a solitary demyelinating lesion

<table>
<thead>
<tr>
<th>NAA/Cr</th>
<th>Acute</th>
<th>1 month</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>4.5</td>
<td>3.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Patient 2</td>
<td>5.2</td>
<td>3.8</td>
<td>5.2</td>
</tr>
<tr>
<td>Patient 3</td>
<td>5.0</td>
<td>3.2</td>
<td>4.7</td>
</tr>
<tr>
<td>Control (mean ± SD)</td>
<td>NAA/Cr 4.9 ± 0.45</td>
<td></td>
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Patient values are compared with those of a similar region of white matter in a group of normal control subjects. Values >2 SD below the control mean are in bold.

Metabolite resonance intensities of NAA, choline (Cho) and creatine (Cr) were determined automatically from peak areas relative to a spline-corrected baseline. The relatively long echo time (TE = 272 ms) used in this study is insensitive to lipid signals, which have short T2, and the observations made should be interpreted in this context. For the purpose of this study, metabolite values were determined for all the voxels located fully outside the demyelinating brain lesions as defined on the T2-weighted MRI and expressed as ratios to Cr in the same voxel. Proton MRSI data were compared with those of similar regions in 15 normal adults. Metabolic changes inside and at the border of these demyelinating lesions, which have been described previously (patients 1, 2 and 3 in De Stefano et al., 1995a), are also reported for comparison. In these regions, the data reported here (Fig. 3) are expressed as ratios to Cr resonance intensities in homologous voxels of normal-appearing contralateral white matter.

Results
For each proton MRSI examination, the mean NAA/Cr metabolite resonance intensity ratios for voxels contralateral
Fig. 2 Proton brain MRI/MRSI examinations of patient 3 performed during the acute phase of a demyelinating disease (left), 1 month later (centre) and 6 months later (right). Conventional proton MRI examinations show a solitary demyelinating lesion that enlarges to involve most of the hemisphere 1 month later (top centre), and then decreases in size on the examination at 6 months (top right). Volumes of interest for spectroscopy are shown by the dotted line in each transverse MRI. Averaged spectra from voxels located in normal-appearing white matter contralateral and homologous to the demyelinating lesion (small squares in top panels) are shown in the bottom panels. Note the decrease in the NAA/Cr resonance intensity 1 month after the acute phase of the disease (bottom centre). Complete recovery of NAA/Cr intensity ratios has occurred at 6 months (bottom right).

and homologous to those co-registered with a solitary demyelinating lesion are reported in Table 1. At the time of the first proton MRSI examination, all patients showed normal metabolite resonance intensity ratios in all voxels in the hemisphere contralateral to demyelinating lesions (Figs 1 and 2 and Table 1), whereas NAA/Cr resonance intensity values were significantly decreased in voxels within the lesions or at their border (40–60 and 20–40%, respectively, relative to the normal control mean) (Fig. 3).

At the MRSI examination performed 1 month later, all patients showed significant decreases in NAA/Cr resonance intensities in voxels of the contralateral hemisphere located in the region homologous to the lesion (Figs 1 and 2 and Table 1), concurrent with further decreases in NAA/Cr resonance intensities in voxels localized inside and at the border of lesions (decreases of 45–75 and 30–70%, respectively) (Fig. 3). Relative to the first MRSI examination, NAA/Cr resonance intensity ratios in this region of the normal-appearing hemisphere decreased by 22, 28 and 35% in patients 1, 2 and 3, respectively. However, other voxels from the normal-appearing hemisphere showed normal NAA/Cr resonance intensities (patient mean = 4.9 ± 0.35; control mean = 4.9 ± 0.45).

A second follow-up examination performed 6 months later...
in all patients showed complete recovery of the reduced NAA/Cr in voxels of the normal-appearing hemisphere (Figs 1 and 2 and Table 1). This was concurrent with partial recovery of the reduced NAA/Cr ratio in voxels located inside and at the border of the demyelinating lesions (relative to the control mean, decreases of 20–50% inside the lesions and of 10–35% at the borders of lesions) (Fig. 3).

Over the follow-up period, measurable resonance intensities of lipid or lactate were not detected in voxels of the brain hemisphere contralateral to lesions and relative resonance intensities of Cho were within normal limits in all patients (mean Cho/Cr in voxels contralateral and homologous to the demyelinating lesion was 0.98 ± 0.1 on the first proton MRSI examination, 1.03 ± 0.1 after 1 month and 0.99 ± 0.1 after 6 months; the control mean in brain white matter was 1.1 ± 0.08).

**Discussion**

The analysis of serial proton MRSI studies from patients who present with large, solitary demyelinating lesions offers special opportunities for definition of the temporal evolution of the biochemical changes occurring in normal-appearing brain remote from focal lesions. In the absence of multifocal lesions, the demonstration that white matter volumes in the normal-appearing hemisphere show metabolic abnormalities suggesting axonal damage or dysfunction can be interpreted as evidence for remote effects of the focal demyelinating lesions. In our study, we found that decreases in NAA/Cr in the hemisphere contralateral to pericallosal lesions were confined to voxels homologous to those that included the demyelinating lesion in the affected hemisphere. These voxels should include the highest density of trans-callosal projections to or from the region of focal demyelination (Nieuwenhuys et al., 1981). Decreases in the relative NAA intensities in these voxels were delayed and smaller than those in the affected hemisphere and appeared to recover completely in all patients over a period of 6 months. This rapid recovery suggests that the observed decreases in NAA contralateral to the lesion arise primarily from axonal dysfunction or volume changes rather than from axonal loss. Such trans-callosal changes could contribute to the altered activation of cortical networks in the normal-appearing hemisphere after the acute injury (Netz et al., 1997; Cao et al., 1998). The possibility of only partial recovery and persistent loss of axons in the contralateral hemisphere due to Wallerian degeneration cannot be excluded, since the residual recovery of NAA in the contralateral volume might then be below the detection sensitivity of the MRSI.

Post-mortem studies have provided evidence for axonal damage in multiple sclerosis both inside and outside of the lesions (Prineas et al., 1993; Ferguson et al., 1997; Trapp et al., 1998). Axons within lesions can change in size, shape and morphology as a result of inflammation and demyelination (Prineas et al., 1993). Metabolic changes leading to the accumulation of amyloid precursor protein and alterations in neurofilament phosphorylation (Ferguson et al., 1997; Trapp et al., 1998) have been shown to occur in nearby areas outside demyelinating lesions. Results reported here extend these post-mortem observations by providing evidence in vivo for reversible metabolic dysfunction in white matter regions that are remote from the demyelinating lesions.

It is possible that trans-synaptic effects also may contribute to the observed decreases in relative NAA concentrations. Potentially reversible depression of neuronal synaptic functions in areas of the central nervous system remote from an acute focal injury are well described as the phenomenon of ‘diaschisis’, which represents metabolic depression in regions distant from but anatomically linked to focal cerebral or cerebellar injury (Baron, 1985; Bowler et al., 1995). Recently, an in vivo proton MRSI study has demonstrated decreases in NAA in the white matter of the cerebellum after a cerebral hemispheric lesion (Fulham et al., 1994). This observation and that of an in vitro spectroscopy study of the visual pathways (Rango et al., 1995) confirm that axonal damage and dysfunction associated with trans-synaptic degeneration can be detected by MR spectroscopy.

In demyelinating diseases, axonal dysfunction remote from visible lesions could also be due to the action of diffusable factors associated with inflammation (Moundjian et al., 1991; Koller et al., 1996; Moreau et al., 1996). However, the observation of focal rather than generalized decreases in NAA/Cr (shown here only in voxels that are contralateral and homologous to the demyelinating lesions) argues against diffusible neurotoxic factors as a cause for the decreases in NAA/Cr seen in these patients.

Using functional MRI, recent studies have demonstrated that relationships between the two cerebral hemispheres are intensified after an acute injury and that the undamaged hemisphere may play a crucial role in recovery after an acute injury (Cao et al., 1998). Thus, the dysfunction of projecting axons seen in our study might be part of the mechanism that leads to increased recruitment of the pre-existing uncrossed pathways or to less inhibition of the hemisphere opposite to the lesion in order to compensate for damage in the crossed pathways after an acute injury. The extent to which decreases in NAA/Cr in brain voxels homologous and contralateral to lesions may reflect part of this or other mechanisms requires further investigation. The use of new, pathologically specific MR techniques may have an important role in this regard.

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References


Axonal dysfunction due to demyelinating lesions


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