Corpus Callosum Axonal Injury in Multiple Sclerosis Measured by Proton Magnetic Resonance Spectroscopic Imaging

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Background: Axonal damage has been observed in normal-appearing white matter (NAWM) for patients with multiple sclerosis (MS).

Objectives: To investigate changes in brain metabolite ratios in a region of normal-appearing corpus callosum (CC) for patients with MS and to test its relationship to changes in other regions of NAWM.

Design and Methods: Data were collected from 24 patients with MS and 15 control subjects. Two-dimensional proton magnetic resonance spectroscopic imaging was performed centered at the CC. Regions of interest from normal-appearing CC were manually segmented using anatomical images. The NAWM outside the CC region was segmented based on the signal intensity in T1- and T2-weighted images.

Results: The N-acetylaspartate–creatine-phosphocreatine ratio was lower in both regions for patients with secondary progressive MS compared with the controls; the N-acetylaspartate–creatine-phosphocreatine was lower only in the normal-appearing CC region for patients with relapsing-remitting MS (P<.001) compared with the controls. The ratio of choline-containing compound compared with the creatine-phosphocreatine ratio was also lower in the region of normal-appearing CC for patients with relapsing-remitting MS (P = .003) compared with the controls. There was a correlation between the N-acetylaspartate–creatine-phosphocreatine ratio in the normal-appearing CC and T1 lesions (r = −0.53, P = .01) for all patients.

Conclusions: The CC was a more sensitive location for depicting axonal injury than other regions of NAWM. A correlation between the reduction of the N-acetylaspartate–creatine-phosphocreatine ratio in the normal-appearing CC and the T1 lesions may suggest that transection of axons in lesions may cause distant axonal damage and/or dysfunction that are expressed and more sensitively detectable in the CC.

Arch Neurol. 2004;61:1081-1086

The development of non-invasive imaging modalities for monitoring disease progression and evaluating response to therapy has become critical for the treatment of patients with multiple sclerosis (MS). While conventional magnetic resonance images (MRIs) provide important information, there remains a need for more sensitive and specific markers of the biological effects of MS. This is particularly true for following up patients with early-stage disease and evaluating differences in factors associated with the various subtypes of MS. Proton magnetic resonance spectroscopic imaging (1H MRSI) is a technique that can be combined with conventional MRI that enables the measurement and quantification of the spatial distribution of brain metabolites such as choline-containing compounds (Cho), creatine-phosphocreatine (Cr), and N-acetylaspartate (NAA). N-acetylaspartate is found mainly in neurons and axons of the mature brain and its intensity level relative to that of Cr has been proposed as an index of axonal damage. Strong motivation for using the NAA/Cr ratio as a marker of disease status in patients with MS has come from observations that axonal loss and/or dysfunction in normal-appearing white matter (NAWM) correlate with clinical disability as measured by the Expanded Disability Status Scale. Although some studies have used single-voxel magnetic resonance spectroscopy (MRS) for studying specific regions of interest (ROIs) in patients with MS, 1H MRSI provides a spatial array of spectral data and is, therefore, particularly valuable for examining variations in metabolite levels in different regions of the brain. Previous studies with 1H MRSI have shown that decreases...
in the NAA/Cr ratio extend beyond the border of visible lesions as well as within the lesions. To be a valuable marker for axonal damage, the NAA/Cr ratio needs to be sensitive to early-stage disease. Although previous studies have shown no significant reduction in the level of NAA in NAWM for patients with clinically isolated syndromes who are at risk of developing MS, the results have been mixed for patients with relapsing-remitting MS (RRMS). It is unclear whether such differences are because of a lack of sensitivity in the techniques being used or because of biological variability in patients with similar disease status.

It is well known that MS lesions have a tendency to cluster in periventricular white matter. This suggests that alterations in the NAA/Cr ratio may also be nonuniformly distributed in NAWM and that regional variations in metabolite levels may be of interest for identifying in vivo measurements of tissue damage. Recent histopathologic studies of the corpus callosum (CC) have reported a significant loss of the total number of axons and the axonal density of fibers relative to nondiseased brain in patients with RRMS and patients with secondary progressive MS (SPMS). If this differential can also be detected using 1H MRSI based on the values of the NAA/Cr ratio, it would represent a noninvasive marker for assessment of early-stage disease and evaluation of response to therapy. This article tested the hypothesis that changes in metabolite ratios in the normal-appearing CC of patients with MS are larger than the changes in other regions of NAWM centered on the CC.

### METHODS

#### STUDY POPULATION

Twenty-four patients with MS from a large cohort followed up at the Multiple Sclerosis Center, University of California, San Francisco, were considered for this study based on the absence of T1- and T2-weighted visible MRI abnormalities in the CC, confirmed by an experienced MS neurologist (D.P.) (12 patients with RRMS and 12 patients with clinically definite MS (SPMS)). If this differential can also be detected using 1H MRSI based on the values of the NAA/Cr ratio, it would represent a noninvasive marker for assessment of early-stage disease and evaluation of response to therapy. This article tested the hypothesis that changes in metabolite ratios in the normal-appearing CC of patients with MS are larger than the changes in other regions of NAWM centered on the CC.

#### MRI AND 1H MRSI EXAMINATION

Magnetic resonance data were acquired using a 1.5-T clinical scanner equipped with a quadrature head coil. Each MRI examination included oblique T2-weighted fast-spin echo (repetition time/echo time [TR/TE], 2000/90 ms; acquisition matrix, 256 × 256 pixels; field of view, 240 × 240 mm2; and 16 contiguous 3-mm-thick sections), axial T2-weighted (TR/TE, 2500/80 ms; acquisition matrix, 192 × 256 pixels; field of view, 180 × 240 mm2; and 48 interleaved 3-mm-thick sections), and axial T1-weighted 3-dimensional spoiled gradient echo (SPGR) (TR/TE, 27/6 ms; flip angle, 40°; acquisition matrix, 192 × 256 × 124 pixels; and field of view, 180 × 240 × 186 mm3) images. The T2-weighted fast-spin echo images were used as a reference for the 1H MRSI acquisition. Two-dimensional chemical shifting imaging was applied with point-resolved spectroscopy volume selection and 1.5-cc nominal spatial resolution using a commercially available pulse sequence (GE Medical System, Milwaukee, Wis). The point-resolved spectroscopy volume was positioned to cover a slab of approximately 160 cc centered at the middle of the CC (central brain [CB]). The 2-dimensional chemical shifting imaging parameters were as follows: TR/TE, 1000/144 ms; phase encoding matrix, 24 × 24 pixels; field of view, 240 × 240 mm2; and 15-mm-thick sections. Automatic shimming and water suppression were applied as part of the data acquisition sequence.

#### POSTPROCESSING

After each examination, the images and raw spectra data were transferred to a SUN Ultra 10 workstation (Sun Microsystems, Calif) for postprocessing. The processing algorithms for the 1H MRSI data were developed in-house and have been described previously. The signals were then quantified by both peak height for the current study because it had been observed that the variation in metabolite ratios obtained from peak areas was larger than for the height. It was anticipated that any difference in line width due to variations in shimming would affect all resonances equally. Voxels corresponding to the anatomical ROIs were determined by resampling the corresponding masks according to the point-resolved spectroscopic selection and chemical shifting imaging phase encoding using in-house software described previously.

The axial T1-weighted SPGR images were resampled to create high-resolution sagittal images to manually segment the CC and exclude visible MS lesions. The ROIs corresponding to the CC were drawn in a conservative manner since it was impossible to find sharp boundaries where it merged into white matter. The CC ROIs were saved as a 3-dimensional axial mask image and resampled to correspond to the T2-weighted fast-spin echo volume image set (NAWMCC). The point-resolved spectroscopic selected volume was composed mainly of white matter with some partial voluming of gray matter and MS lesions. Tissue with the intensity characteristics of NAWM was segmented based on the high-resolution T1-weighted SPGR images with the exclusion of the ROIs corresponding to the CC and of regions that had intensity characteristics of cerebral spinal fluid and MS lesions on corresponding T2-weighted images (NAWMpCC). Masks with constant values in the NAWMCC and NAWMpCC were generated for analysis of the corresponding spectral intensities. Figure 1 shows the point-resolved spectroscopic selected volume, NAWMCC, and NAWMpCC ROIs for a control and for a patient with SPMS.

The NAWMpCC voxels were determined by including only those that had more than 10% overlap with the region corresponding to NAWM for all subjects. To avoid variations in intensity because of voxels at the edge of the point-resolved spectroscopic selected volume, only the central 8 × 10 voxels of the chemical shifting imaging array were used to represent those metabolite levels. The NAWMCC voxels were identified by determining which had more than 20% overlap with the CC region for all subjects. Owing to the limited spatial resolution of the 1H MRSI used in the current study, some degree of partial voluming with gray matter would be expected for certain NAWMCC voxels, particularly for patients with callosal atrophy. Findings from a previous study from our laboratory showed that NAA and Cr levels were proportionally higher in the cortical gray matter than white matter. Based on this finding, we believe that the degree of partial voluming with gray matter in the NAWMCC voxels would not adversely affect the NAA/Cr ratio for all subjects.

Regions of interest corresponding to T1 lesions were drawn based on semiautomated threshold with manual editing on the axial T1-weighted 3-dimensional SPGR volume by an experienced MS neurologist (D.P.) including hypointense relative to the white matter as well as isointense to the gray matter. The

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The mean age was 45.1 years for all patients and 43.2 years for controls. The mean age, disease duration, Expanded Disability Status Scale score, and T1 lesion load for all subjects are listed in Table 1.

**METABOLITE LEVELS IN THE ROIS**

To avoid the effect of differential coil loading on the signal from different subjects, variations in metabolite ratios in the entire point-resolved spectroscopic volume (CB region), NAWM _CB−CC_, and NAWM _CC_ ROIs were compared in this study. Note that the CC and NAWM ROIs considered in this study were chosen to exclude visible T1 (3-dimensional SPGR) and T2 (SE) lesions. This had the effect of reducing the mean number of voxels in the NAWM _CB−CC_ ROIs from 14.3 in the controls to 10.3 in the the subjects, variations in metabolite ratios were considered to exclude visible T1 (3-dimensional SPGR) and T2 (SE) lesions. This had the effect of reducing the mean number of voxels in the NAWM _CB−CC_ ROIs from 14.3 in the controls to 10.3 in the the patients with RRMS and 8.3 in the patients with SPMS. Metabolite levels from the NAWM _CB−CC_ voxels from 1 patient with RRMS and 4 patients with SPMS were excluded from the analysis because there were fewer than 3 voxels satisfying the definition of having more than 90% overlap with the corresponding ROIs. The mean number of voxels in the NAWM _CC_ ROIs were 6.2 in the controls, 6.5 in the patients with RRMS, and 5.5 in the patients with SPMS.

There was a significant reduction in the NAA/Cr ratio for the patients with SPMS (P = .01) compared with the controls (Table 2). There was no difference in the mean value for the patients with RRMS. However, if we consider the metabolite levels from the entire CB rather than just the NAWM, the results are confused by the inability to differentiate between metabolite levels in gray matter, white matter, or lesions and to account for the effects of atrophy. The NAA/Cr ratio was slightly reduced but did not reach significance for the patients with SPMS (P = .35) compared with the controls in the NAWM _CB−CC_ ROIs (Table 3). There was no difference in the mean value for patients RRMS in the same region.

In healthy controls, it was observed that there were significantly higher NAA/Cr _P<.001_, NAA/Cho _P=.03_, and Cho/Cr _P=.05_ ratios in the NAWM _CC_ compared with the NAWM _CB−CC_. There were highly significant reductions of the NAA/Cr ratio derived from the NAWM _CC_ for both patient groups compared with the controls (P = .001 for patients with RRMS and _P<.001_ for patients with SPMS), and reduction in the Cho/Cr ratio for patients with RRMS (P = .003) and patients with SPMS with marginal significance (P = .09) compared with the controls (Table 3).

**RESULTS**

**CORRELATIONS BETWEEN T1 LESION LOAD AND MRSI PARAMETERS**

There was a significant correlation between T1 lesion load and the NAA/Cr ratio in the entire CB (r = −.046, _P = .03_) for all patients combined. Similar correlation was observed in the NAWM _CC_ region (r = −.53, _P = .01_) but not in the NAWM _CB−CC_ (r = −.40, _P = .10_).

**COMMENT**

This study has addressed variations in metabolite levels in the CC compared with other regions of white matter.
Table 1. Clinical Characteristics for Individual Subgroups*

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, y</th>
<th>Disease Duration, y</th>
<th>EDSS Score</th>
<th>T1 Lesion Load, cc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects (n = 15)</td>
<td>43.2 (9.1) [23.6-57.2]</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with RRMS (n = 12)</td>
<td>41.4 (10.0) [22.2-59.7]</td>
<td>8.4 (7.0) [4.5-30.2]</td>
<td>2.0 (1.4) [0.0-4.5]</td>
<td>1.4 (2.3) [0.01-7.6]</td>
</tr>
<tr>
<td>Patients with SPMS (n = 12)</td>
<td>48.9 (6.8) [39.0-59.0]</td>
<td>18.8 (7.7) [6.7-29.2]</td>
<td>6.2 (1.1) [3.5-8.0]</td>
<td>3.3 (4.5) [0.02-13.6]</td>
</tr>
</tbody>
</table>

Abbreviations: EDSS, Expanded Disability Status Scale; NA, not applicable; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
*Data are given as the mean (SD) [range].

Table 2. Age-Adjusted Mean Metabolite Ratios for All Patients With Multiple Sclerosis and Control Subjects Derived From the Central Brain Regions*

<table>
<thead>
<tr>
<th>Group</th>
<th>NAA/Cr Ratio</th>
<th>P Value</th>
<th>NAA/Cho Ratio</th>
<th>P Value</th>
<th>Cho/Cr Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>2.00 (0.03)</td>
<td>NA</td>
<td>1.59 (0.05)</td>
<td>NA</td>
<td>1.28 (0.03)</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with RRMS</td>
<td>1.95 (0.04)</td>
<td>.29</td>
<td>1.59 (0.06)</td>
<td>.96</td>
<td>1.25 (0.04)</td>
<td>.54</td>
</tr>
<tr>
<td>Patients with SPMS</td>
<td>1.87 (0.04)</td>
<td>.01</td>
<td>1.55 (0.06)</td>
<td>.58</td>
<td>1.25 (0.04)</td>
<td>.62</td>
</tr>
</tbody>
</table>

Abbreviations: Cho, choline-containing compounds; Cr, creatine-phosphocreatine; NA, not applicable; NAA, N-acetylaspartate; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
*Data are given as the least square mean (SE) with age adjustment.

Table 3. Age-Adjusted Mean Metabolite Ratios for All Patients With Multiple Sclerosis and Control Subjects Derived From the NAWM in the NAWMCB−CC and NAWMCC ROIs*

<table>
<thead>
<tr>
<th>Group</th>
<th>NAWMCB−CC NAA/Cr Ratio</th>
<th>P Value</th>
<th>NAWMCB−CC NAA/Cho Ratio</th>
<th>P Value</th>
<th>NAWMCB−CC Cho/Cr Ratio</th>
<th>P Value</th>
<th>NAWMCC NAA/Cr Ratio</th>
<th>P Value</th>
<th>NAWMCC NAA/Cho Ratio</th>
<th>P Value</th>
<th>NAWMCC Cho/Cr Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>2.12 (0.05)</td>
<td>NA</td>
<td>1.54 (0.06)</td>
<td>NA</td>
<td>1.39 (0.04)</td>
<td>NA</td>
<td>2.39 (0.06)</td>
<td>NA</td>
<td>1.64 (0.06)</td>
<td>NA</td>
<td>1.48 (0.05)</td>
<td>NA</td>
</tr>
<tr>
<td>Patients with RRMS</td>
<td>2.11 (0.06)</td>
<td>.91</td>
<td>1.54 (0.07)</td>
<td>.99</td>
<td>1.38 (0.05)</td>
<td>.90</td>
<td>2.06 (0.07)</td>
<td>.001</td>
<td>1.64 (0.06)</td>
<td>.98</td>
<td>1.26 (0.05)</td>
<td>.03</td>
</tr>
<tr>
<td>Patients with SPMS</td>
<td>2.03 (0.08)</td>
<td>.35</td>
<td>1.47 (0.08)</td>
<td>.53</td>
<td>1.41 (0.06)</td>
<td>.83</td>
<td>2.01 (0.08)</td>
<td>&lt;.001</td>
<td>1.49 (0.07)</td>
<td>.11</td>
<td>1.35 (0.06)</td>
<td>.09</td>
</tr>
</tbody>
</table>

Abbreviations: Cho, choline-containing compounds; Cr, creatine-phosphocreatine; NA, not applicable; NAA, N-acetylaspartate; NAWM, normal-appearing white matter; NAWMCB−CC, NAWM of the central brain outside the corpus callosum; NAWMCC, NAWM of the corpus callosum; ROIs, regions of interest; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.
*Data are given as the least square mean (SE) with age adjustment.

for the healthy control and MS population. The first finding was that significantly higher NAA/Cr, NAA/Cho, and Cho/Cr ratios were observed in the CC compared with other regions of white matter from the healthy controls. This finding is consistent with previous results from the literature and is thought to be due to the increased axonal density in the highly ordered region of the brain. The second finding was that both patients groups showed a significant decrease of the NAA/Cr ratio in the normal-appearing CC compared with both controls and their own NAWM. Although results from our research group showed a small reduction of the NAA/Cr ratio in a small group of patients with early-stage RRMS with a mean disease duration of 5 years, this was not statistically significant in whole supratentorial and CB regions (P = .36 and P = .33, respectively). The approach suggested in our study, which analyzes differences in metabolite levels in the CC compared with levels from NAWM in the same individual, may assist in reducing the effects of biological variability and, hence, provide a more sensitive assay of axonal damage. There was a trend to a reduced NAA/Cr ratio in voxels from NAWM for patients with SPMS but not for patients with RRMS. In conjunction with findings from our current NAWMCC, this suggests that the differences in the ability to detect changes in the NAA/Cr ratio in previous studies may be explained by variations in the region of the brain studied and not to intrinsic differences between populations. This may indicate that the normal-appearing CC is a more sensitive region for detecting axonal damage owing to densely packed unidirectional long fiber that may accentuate the reduction in the NAA/Cr ratio compared with other regions of white matter.

Pelletier et al showed that patients with early RRMS with mild disability had significant callosal atrophy and its relationship with brain T2 lesion volume and functional transfer indexes. These findings support our hypothesis that demyelinating white matter lesions induce axonal loss and Wallerian degeneration that can be detected in the CC. In our study, however, we investigated damages in the NAWM regions caused by remote...
distant MS lesions, not MS plaque in the CC. A moderate but significant correlation between T1 lesions and the NAA/Cr ratio was observed in the normal-appearing CC for all patients with MS. This may be explained by the transection of axons in lesions causing distant axonal damage and/or dysfunction that are expressed in the normal-appearing CC.\(^\text{13,21}\) Such a moderate correlation coefficient can be explained by the fact that some transected axons in lesions simply do not cross the CC. The lack of correlation for the same parameters in the NAWM outside the CC may support our findings that improved sensitivity to detect metabolite changes induced by distant MS lesions was observed in the CC.

Our study also showed that there was reduction of the Cho/Cr ratio within the CC for patients with MS compared with healthy controls. These results may suggest some degree of decreased cellularity.\(^\text{22}\) The stability of the Cho/Cr ratio in the NAWM voxels is consistent with previous studies.\(^\text{14,23}\) The similarity between the NAA/Cho ratio in the CC for the healthy controls and all subgroups of patients with MS is most likely explained by a concomitant reduction in the levels of both NAA and Cho.

We should mention that the point-resolved spectroscopic volume acquired with the 2-dimensional \(^1\)HMRSI sequence used in our study did not always cover all of the CC. This means that the number of voxels used to calculate the metabolite levels and, thus, the reliability of the estimated metabolite ratios could be increased by the use of a 3-dimensional \(^1\)HMRSI technique. Although we are technically capable of performing such data acquisitions, we designed the current study to include the commercially available 2-dimensional \(^1\)HMRSI sequence because we wanted to develop a robust method that could potentially be applied routinely at multiple institutions. Another conservative aspect of our study was the decision to manually segment the CC rather than letting a clinician visually identify the voxels corresponding to this region. This helped us to be objective in interpreting our results, but when we looked at the voxels that were selected using our segmentation procedure, it was clear that manual selection by an individual who was familiar with neuroanatomy would probably have given similar selection of voxels. As the availability of clinical packages for obtaining and analyzing 3-dimensional \(^1\)HMRSI increases it should be possible to simplify the analysis so that metabolite ratios from the CC can be obtained directly on the scanner console and the statistical power of the test can be improved by including a larger number of voxels for each patient. This should provide a sensitive method for routinely obtaining a quantitative measure of axonal damage in patients with MS that can be applied at multiple institutions and used for following disease progression or response to therapy.

CONCLUSIONS

This study demonstrates that it is possible to measure significant axonal loss and/or dysfunction in the normal-appearing CC in the brain of patients with MS noninvasively using a commercially available 2-dimensional \(^1\)HMRSI sequence. Significant reductions of the NAA/Cr and Cho/Cr ratios were observed in the normal-appearing CC region compared with the other NAWM regions. This suggests that the CC may be a sensitive location for depicting axonal injury in patients with MS, especially in the early stage of the disease. The \(^1\)HMRSI techniques that were used can be expected to provide the basis for a widely available and robust measure of axonal damage.

Accepted for publication February 25, 2004.

Author contributions: Study concept and design (Drs Oh, Pelletier, and Nelson); acquisition of data (Drs Oh, Pelletier, and Nelson); analysis and interpretation of data (Drs Oh, Pelletier, and Nelson); drafting of the manuscript (Dr Oh); critical revision of the manuscript for important intellectual content (Drs Oh, Pelletier, and Nelson); statistical expertise (Dr Oh); obtained funding (Drs Pelletier and Nelson); administrative, technical, and material support (Drs Oh, Pelletier, and Nelson); study supervision (Drs Pelletier and Nelson).

This study was supported in part by grants RG2655B6/1 from the National Multiple Sclerosis Society, New York, NY, and R01 NS39529, from the National Institutes of Health, Bethesda, Md.

Dr Oh is a National Multiple Sclerosis Society Postdoctoral Fellowship awardee.

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