A Quantitative Study of Water Diffusion in Multiple Sclerosis Lesions and Normal- Appearing White Matter Using Echo-Planar Imaging

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Objectives: We used an optimized echo-planar pulse sequence for isotropically weighted diffusion imaging (1) to measure mean diffusivity ($D_\text{æ}$) in lesions and normal-appearing white matter (NAWM) in the entire brain from patients with mildly disabling relapsing-remitting multiple sclerosis (MS), (2) to compare the $D_\text{æ}$ of NAWM from patients with that of white matter from normal controls, (3) to evaluate whether lesions classified on the basis of their appearance on enhanced T1-weighted scans have different $D_\text{æ}$, and (4) to investigate the relationship between diffusion changes in lesions and NAWM.

Methods: Dual-echo and diffusion-weighted scans were obtained from 35 patients with relapsing-remitting MS and 24 sex- and age-matched normal controls. Postcontrast T1-weighted images were also obtained from the patients. After creating $D_\text{æ}$ maps and image coregistration, $D_\text{æ}$ values were measured for MS lesions larger than 5 mm and for 22 NAWM areas from each subject.

Results: All NAWM areas studied had significantly higher $D_\text{æ}$ in patients than in controls. A total of 173 lesions were identified on the dual-echo scans from patients. The average $D_\text{æ}$ for these lesions was significantly higher than that of NAWM. Twenty-two lesions were enhancing and 60 were classified as T1-hypointense. No significant difference in $D_\text{æ}$ values was found between enhancing and nonenhancing lesions, while the average $D_\text{æ}$ of T1-hypointense lesions was significantly higher than the average $D_\text{æ}$ of T1-isointense lesions. There was no significant correlation between the average $D_\text{æ}$ in lesions and NAWM.

Conclusions: This study shows that diffusion-weighted imaging is able to identify MS lesions with severe tissue disruption. It also shows that widespread increased diffusion can be measured in the NAWM from patients with MS, and suggests that such changes are, at least partially, independent of larger abnormalities.

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In multiple sclerosis (MS), conventional magnetic resonance imaging (MRI) has proved to be sensitive in detecting lesions and their changes over time. However, conventional MRI is not without relevant limitations. These include the lack of specificity to the heterogeneous pathologic substrates of MS lesions and the inability to detect subtle abnormalities in the so-called normal-appearing white matter (NAWM).

Diffusion-weighted imaging (DWI) is a promising tool for overcoming the limitations of conventional MRI. It provides a unique form of magnetic resonance contrast that enables diffusion of water molecules to be measured and, as a consequence, provides information about the orientation, size, and geometry of brain structures. If water molecules are free to move in any direction from a certain position, they will spread out over time, and the magnitude of the distance moved is characterized by the molecular self diffusion coefficient. Measurements of water diffusion in vivo can be easily performed by MRI in terms of an apparent diffusion coefficient (ADC), which incorporates the effects of all the intravoxel incoherent motions, including molecular diffusion and microcirculation in capillaries. Cellular structures in the central nervous system restrict water molecular motion, and therefore the distance moved over time seems reduced. Hence, the ADC measured in vivo is always lower than the true diffusion coefficient of water at body temperature. Pathologic processes that modify tissue integrity, thus reducing “restricting” barriers, can result in increased ADC. Because some cellular structures such as axons are aligned on the scale of an image pixel, the restriction and ADC are also dependent on the direction in which diffusion is measured, thus making comparison of ADC.
PATIENTS AND METHODS

SUBJECTS

We studied 35 patients with clinically definite, relapsing-remitting MS13,16 (20 women and 15 men). Their mean (SD) age was 28.0 (4.8) years; median duration of the disease, 3.5 years (range, 1-8 years); and median Expanded Disability Status Scale score,17 1.5 (range, 1.0-3.0). To be included in the study, all patients had to have been untreated with immunosuppressive or immunomodulatory drugs for at least 1 year before study entry. They also should not have had relapses and steroid treatment in the 3 months preceding the study. Twenty-four healthy volunteers (15 women and 9 men; mean [SD] age, 29.0 [3.7] years) served as controls. Local ethical committee approval and written informed consent from all the subjects were obtained before study initiation.

MAGNETIC RESONANCE IMAGING

Brain MRI scans were obtained from patients and controls using a scanner operating at 1.5 T. During a single session, the following scans were performed without moving the subject from the scanner: (1) dual-echo conventional spin echo (repetition time [TR], 3300 milliseconds; first echo echo time [TE], 30 milliseconds; second echo TE, 80 milliseconds; number of acquisitions, 1); (2) spin echo echoplanar (interecho spacing, 0.8; TE, 160 milliseconds), collecting 1 T2-weighted image and 3 DWIs for each slice, with identical diffusion-encoding waveforms, designed to give an attenuation dependent only on the trace of the diffusion tensor (Tr[D]) and a resulting b factor of 289 s/mm², in each of the read, phase-encoded, and slice-selection directions (isotropically weighted imaging); and (3) T1-weighted conventional spin echo (TR, 680 milliseconds; TE, 17 milliseconds; number of acquisitions, 2) 10 minutes after the injection of 0.1-mmol/kg gadolinium-DTPA (this sequence was not obtained from controls).

For the dual-echo and the T1-weighted scans, 24 contiguous interleaved axial slices were acquired with a 5-mm slice thickness, a 256×256 matrix, and a 250×250-mm field of view, giving an in-plane spatial resolution of about 1×1 mm. The set of slices for the echo-planar DWIs was the same used for the dual-echo, with a 128×128 matrix and a 250×250-mm field of view. The manufacturer’s own phase correction and regridding algorithm were used before Fourier transformation and interpolation to a 256×256 image matrix.

IMAGE ANALYSIS AND POSTPROCESSING

Lesions were identified on the proton density–weighted scans and marked by agreement on the hard copies by 2 observers who were unaware of the patients’ clinical status. T2-weighted images were always used to increase confidence in lesion identification. To minimize partial volume effects, only lesions with a diameter greater than 5 mm underwent subsequent analysis. Using postcontrast T1-weighted scans, we classified these lesions as enhancing or nonenhancing, according to published criteria.16 Nonenhancing lesions were classified as T1 isointense or hypointense. T1 hypointensity was defined as reduced lesion signal intensity with respect to surrounding NAWM. A single observer, unaware of lesion classification, displayed the dual-echo images on a computer screen and, using marked hard copies as a reference, outlined these lesions on the proton density–weighted images and measured lesion volumes using a semi-automated segmentation technique based on local thresholding.18 The outlined regions of interest were then mapped onto the coregistered D maps, and the areas and D of each lesion measured. Coregistration of images was performed using a surface-matching technique, based on mutual information.20 Mean diffusivity maps were obtained as follows: The average of the 3 DWIs was calculated to improve the signal-to-noise ratio. Images were then realigned to correct for misregistration due to eddy currents. Finally, an image of the \( \bar{D} (\text{Tr}[D]/3) \) was produced by performing the following calculation on a pixel-by-pixel basis:

\[
\bar{D} = (1/3b)\ln (S_p/S_i)
\]

where \( S_p \) is the average DW signal intensity and \( S_i \) is the T2-weighted signal intensity (with \( b = 0 \)) (Figure 1). The same segmentation technique used for measuring lesion volumes on proton density–weighted images.

Mean diffusivity values of NAWM in different brain regions were also studied; the NAWM areas were selected on the dual-echo scans. They did not have to have adjacent lesions either in the same slice or in the slices above and below. Whenever possible, square regions of interest of uniform size (3×3 pixels) were placed bilaterally in the white matter of the following areas: the pons, the cerebellar hemispheres, the internal capsules, the anterior and posterior portions of the corpus callosum, and the temporal, parietal, occipital, and frontal lobes (in regions far from ventricles and cortical gray matter). Two regions of interest were also placed close to the anterior and posterior parts of the body of the lateral ventricles, and one close to the cortical gray matter of the frontal lobe. The outlined NAWM regions were then transferred onto the coregistered D maps. Using the same method, the \( \bar{D} \) values of the same brain regions were measured from controls.

STATISTICAL ANALYSIS

A 2-tailed t test for not-paired data was used to compare (1) the \( \bar{D} \) of the NAWM from patients with that of the white matter from controls and (2) the \( \bar{D} \) of the NAWM with that of lesions from patients. The Mann-Whitney test was used to compare (1) the \( \bar{D} \) and area of enhancing vs nonenhancing lesions and (2) the \( \bar{D} \) and area of T1-hypointense vs T1-isointense lesions. Univariate correlations were assessed using the Spearman rank correlation coefficient.

values meaningless without taking into account the measurement direction. One measurement of diffusion that is independent of the orientation of structures is provided by measuring the ADC in 3 orthogonal directions, and then averaging the results to form the mean diffusivity (\( \bar{D} \)). Preliminary studies showed that it is possible to quantify the tissue damage in MS lesions visible on T2-
and orientation of water spaces) that are inaccessible to other MRI techniques. The pathologic elements of MS can alter the permeability or geometry of structural barriers to water diffusion in the brain. Therefore, although DWI has been mostly used for the early detection of cerebral ischemia, a promising application of the technique is its use to quantify the structural damage in MS. In this study, we measured $D$ values of lesions and different NAWM areas using an optimized EPI-DWI sequence to elucidate the nature of brain damage in patients with MS. We assessed patients with early relapsing-remitting MS and a very narrow range of disability, and these are likely to be the main reasons why a correlation between $D$ values and disease duration or level of disability was not found. Although this issue warrants further investigation, we believe that our results confirm the potential of DWI to elucidate in vivo the nature of the damage in MS lesions and NAWM and provide important information about the pathophysiological characteristics of MS during its early phases.

We found that the average diffusivity is higher in the NAWM from patients with mildly disabling relapsing-remitting MS than in the white matter from controls. Although this indicates a net loss of structural barriers to water molecular motion in the NAWM, we can only speculate on the possible pathologic substrates of this finding. Subtle changes are known to occur in the NAWM from patients with MS, including diffuse astrocytic hyperplasia, patchy edema, perivascular infiltration, gliosis, abnormally thin myelin, and axonal loss. Since

**RESULTS**

Diffusion-weighted images were of good quality for all patients and controls and were not affected by motion artifacts or geometric distortions. The average $D$ of the 747 NAWM areas from patients was $0.821 \times 10^{-3}$ mm$^2$/s (SD, $0.18 \times 10^{-3}$ mm$^2$/s) and was significantly higher ($P<.001$) than that of the 528 white matter areas from controls ($0.727 \times 10^{-3}$ mm$^2$/s [SD, $0.09 \times 10^{-3}$ mm$^2$/s]). In the Table, the average $D$ values and SDs of the NAWM for each of the anatomical regions studied from patients and controls are reported. Patients with MS had significantly higher $D$ values in all the areas studied.

A total of 173 lesions were identified on the dual-echo scans from patients. The average $D$ for these lesions was $1.085 \times 10^{-3}$ mm$^2$/s (SD, $0.10 \times 10^{-3}$ mm$^2$/s) and was significantly higher ($P<.001$) than the average $D$ of NAWM. Twenty-two lesions were enhancing, and 60 were classified as T1-hypointense. No significant difference in $D$ was found between enhancing (mean [SD], $1.039 \times 10^{-3}$ mm$^2$/s) and nonenhancing lesions ($1.040 \times 10^{-3}$ mm$^2$/s), while the average $D$ of T1-hypointense lesions ($1.115 \times 10^{-3}$ mm$^2$/s) was significantly higher ($P<.001$) than the average $D$ ($0.995 \times 10^{-3}$ mm$^2$/s) of T1-isointense lesions (Figure 2). Mean lesion volumes were similar for enhancing and nonenhancing and for T1-hypointense and T1-isointense lesions. There was no significant correlation between the average $D$ values in lesions and NAWM ($r=0.18$). Moderate correlations were found between the average $D$ in NAWM and the T2-hyperintense and T1-hypointense lesion volumes ($r=0.4$; $P=0.03$). There were no significant correlations between average $D$ values in lesions and NAWM and patients’ age, disease duration, and Expanded Disability Status Scale level.

**COMMENT**

Diffusion-weighted imaging is able to probe structural properties of tissue (including the size, shape, integrity, and orientation of water spaces) that are inaccessible to other MRI techniques. The pathologic elements of MS can alter the permeability or geometry of structural barriers to water diffusion in the brain. Therefore, although DWI has been mostly used for the early detection of cerebral ischemia, a promising application of the technique is its use to quantify the structural damage in MS. In this study, we measured $D$ values of lesions and different NAWM areas using an optimized EPI-DWI sequence to elucidate the nature of brain damage in patients with MS. We assessed patients with early relapsing-remitting MS and a very narrow range of disability, and these are likely to be the main reasons why a correlation between $D$ values and disease duration or level of disability was not found. Although this issue warrants further investigation, we believe that our results confirm the potential of DWI to elucidate in vivo the nature of the damage in MS lesions and NAWM and provide important information about the pathophysiological characteristics of MS during its early phases.

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**Figure 1.** Axial magnetic resonance images of the brain from a patient with multiple sclerosis. In the conventional proton density–weighted image, (A), several hyperintense lesions are visible. The echo-planar pulse sequence used for measuring mean diffusivity (B) collects a T2-weighted, (B) and 3 identical isotropic diffusion–weighted images per slice. The average of the 3 diffusion-weighted images is then calculated, (C), to derive, on a pixel-by-pixel basis, the quantitative map, (D) of the $D$. [Image 318x482 to 546x746]
“inflammatory” changes and gliosis could potentially restrict water molecular motion, we believe that myelin and axonal loss are the most likely contributors to the increased $D$ we observed in the NAWM. Results from postmortem, magnetic resonance spectroscopy, and magnetization transfer imaging (MTI) studies support the concept that tissue destruction can occur in the NAWM of patients with MS and suggest that this might contribute to patients’ clinical status. In addition to previous observations, this study provides systematic evaluation of different brain regions and indicates that NAWM changes in MS are widespread. Interestingly, such abnormalities seem to be more severe in sites such as the corpus callosum, the internal capsules, or the periventricular and subcortical regions, where macroscopic MS lesions are also usually located.

In our sample, the $D$ of NAWM was not significantly correlated with the $D$ of macroscopic lesions and was only moderately correlated with lesion extent on T2- and T1-weighted images. This suggests that subtle NAWM changes are not merely the result of wallerian degeneration of axons transversing larger lesions, but they more likely represent small focal abnormalities beyond the resolution of conventional scanning and independent of larger lesions. This agrees with the demonstration that tiny MS lesions can be seen when using high field magnets or thin slices. If confirmed, this finding indicates that measuring $D$ in NAWM and lesions may provide partially independent and complementary data for assessing brain abnormalities from patients with MS.

Mean diffusivity values are highly variable in MS lesions and higher than those from NAWM. This indicates that variable degrees of tissue damage occur within MS lesions, and confirms the results of previous studies obtained from patients in more advanced phases of the disease. We measured the highest $D$ in T1-hypointense lesions. Previous studies showed that T1-hypointense lesions are those in which severe tissue loss has occurred and their extent is correlated with disease progression in patients with secondary progressive MS. Although postmortem studies correlating histopathologic characteristics and DWI changes are needed, the observation that T1-hypointense lesions have the highest $D$ suggests that axonal loss and demyelination are the most likely pathologic substrates of increased $D$ in MS. We did not find any difference in the $D$ between enhancing lesions and nonenhancing lesions. Previous studies achieved conflicting results on this aspect. A navigated spin-echo diffusion study found no difference in $D$ between enhancing and nonenhancing lesions, but other studies found higher diffusivity in enhancing or acute lesions. However, in one study only 2 acute lesions were studied, while in the other, although water diffusion was measured from 15 enhancing lesions, they were all from a small group of patients with different clinical courses. In acute (enhancing) lesions, vasogenic edema, demyelination, and axonal loss can determine increased $D$, while remyelination, presence of inflammatory cells, and myelin breakdown products can create “restricting” barriers. In chronic (nonenhancing) lesions, tissue loss can increase $D$ values, while gliosis or low-grade inflammatory processes can act as restricting barriers. This

### Table: Average (SD) Mean Diffusivity ($D$) in Different White Matter Areas From Controls and Normal-Appearing White Matter Areas From Patients With Multiple Sclerosis (MS)*

<table>
<thead>
<tr>
<th>Brain Area</th>
<th>Controls</th>
<th>No. of Areas Studied</th>
<th>$D \times 10^{-3}$ mm$^2$/s</th>
<th>Patients With MS</th>
<th>No. of Areas Studied</th>
<th>Median Difference in $D$ Between Controls and Patients</th>
<th>$P^\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All areas</td>
<td>0.727 (0.08)</td>
<td>528</td>
<td>0.747 (0.18)</td>
<td>747</td>
<td>6.5</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.690 (0.21)</td>
<td>48</td>
<td>0.701 (0.26)</td>
<td>70</td>
<td>1.9</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Pons</td>
<td>0.695 (0.12)</td>
<td>48</td>
<td>0.725 (0.43)</td>
<td>70</td>
<td>3.2</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>0.700 (0.14)</td>
<td>48</td>
<td>0.762 (0.41)</td>
<td>70</td>
<td>8.2</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>0.699 (0.12)</td>
<td>48</td>
<td>0.738 (0.37)</td>
<td>70</td>
<td>5.4</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Parietal lobe</td>
<td>0.701 (0.14)</td>
<td>48</td>
<td>0.754 (0.47)</td>
<td>70</td>
<td>2.2</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>0.697 (0.14)</td>
<td>48</td>
<td>0.721 (0.31)</td>
<td>70</td>
<td>9.8</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Subcortical areas</td>
<td>0.751 (0.12)</td>
<td>48</td>
<td>0.916 (0.15)</td>
<td>65</td>
<td>25.0</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Posterior PV areas</td>
<td>0.795 (0.07)</td>
<td>48</td>
<td>0.939 (0.17)</td>
<td>64</td>
<td>13.2</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Anterior PV areas</td>
<td>0.716 (0.12)</td>
<td>48</td>
<td>0.813 (0.10)</td>
<td>67</td>
<td>13.7</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0.763 (0.13)</td>
<td>48</td>
<td>1.128 (0.33)</td>
<td>66</td>
<td>37.5</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Internal capsule</td>
<td>0.787 (0.09)</td>
<td>48</td>
<td>0.865 (0.09)</td>
<td>67</td>
<td>9.7</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

*PV indicates periventricular.
†From t test for not-paired data.
suggests that DWI gives complementary information to T1-weighted images, with the advantage that it provides quantitative measurements. Our results also support the concept that enhancing and nonenhancing lesions have highly heterogeneous pathologic substrates with different effects on water molecular motion.

In conclusion, our results show that severe structural changes can occur in MS lesions from the early phases of the disease. They also indicate that changes in water diffusivity, although milder, can be detected in several areas of NAWM and are more pronounced in those areas that are typical sites of MS macroscopic lesions. These observations call for therapeutic interventions to prevent tissue loss in MS early in the course of the disease. Longitudinal studies are now warranted to assess whether an increased proportion of lesions with markedly increased $D$ or larger portions of NAWM with abnormal $D$ are associated with an increased risk of a subsequent unfavorable evolution of the disease.

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