ABSTRACT

Multiple sclerosis is a complex disease involving inflammatory and neurodegenerative processes. Imaging techniques that can quantitate the nature and the extent of tissue damage as well as reversible changes (e.g., remyelination and repair) will be essential for therapeutic trials with neuroprotective agents. Newer magnetic resonance imaging (MRI) measures that visualize the pathology of the disease also may improve the modest correlation between MRI and clinical disability. Conventional MRI (cMRI), using T1- and T2-weighted imaging sequences, can accurately determine the number of new contrast-enhancing lesions (acute blood-brain barrier disruptions) and total burden of disease (T2 lesion load); however, these methods lack pathologic specificity. cMRI also fails to measure microscopic disease in the normal-appearing white matter and gray matter structures. To address this limitation, techniques that measure diffuse disease are being implemented. These include cerebral atrophy measures, proton magnetic resonance spectroscopy (MRS), magnetization transfer imaging, and diffusion tensor imaging. With the exception of MRS, which estimates axonal density by measuring the neuronal-specific marker, N-acetyl aspartate, none of these imaging measures are specific for axonal loss. However, they have provided valuable insights into the extent of tissue damage and the pathology of this complex disease process.


Our concept of multiple sclerosis (MS) as a purely demyelinating disease was revolutionized when immunohistochemistry studies using confocal microscopy showed axonal transection in acute and chronic lesions in pathologic specimens of MS brains.1 The number of transected axons in MS lesions is estimated at 11,000 ovoids/mm3. As a result of these findings, it is currently believed that axonal damage rather than demyelination is responsible for permanent neurologic impairment.

The disease process in MS is considered as having 2 distinct pathologic phases. In early disease or relapsing-remitting MS (RRMS), the disease is characterized by inflammation and clinical relapses. Focal inflammatory events, causing blood-brain barrier breakdown, are imaged on magnetic resonance imaging (MRI) as contrast-enhancing lesions (CEL), which generally persist for 1 month or less. Although enhancement will cease, CELs persist as hyperintense focal lesions on T2-weighted images. Patients with RRMS generally evolve into a secondary progressive (SPMS) stage of disease within 10 to 15 years. In SPMS the predominant disease pathology is neurodegeneration. New inflammatory lesions are reduced, but the disease becomes more diffuse involving normal-appearing white matter (NAWM) and gray matter (GM) structures. On conventional MRI (cMRI), the best techniques for measuring neurodegeneration are T1 black holes and cerebral atrophy. Newer MRI techniques (e.g., proton magnetic resonance spectroscopy [MRS], magnetization transfer imaging [MTI], and diffusion tensor imaging [DTI]) also are being used to measure focal and diffuse disease.

The relationship between the inflammatory lesions and subsequent neurodegeneration is unclear. Axonal loss and cerebral atrophy can be detected at even the earliest stages of the disease, which leads some to suggest that the disease is primarily a diffuse process.
throughout the white matter, and inflammatory lesions are inconsequential. Others believe inflammatory lesions are the inciting events that precede the eventual axonal damage. Evidence for the latter hypothesis is provided again by confocal microscopy studies, which demonstrated that the axonal loss in NAWM (17 axonal transections/mm³) is miniscule compared to the axonal damage within visible lesions.¹

To address the question of which MRI methods are useful measures of neuroprotection and repair, we need to consider and quantitate 3 events. First, we need to demonstrate that ongoing inflammation is halted. Second, the extent of tissue damage must be assessed. Third, new axonal growth and/or remyelination must be measurable. The MRI technique should therefore be capable of measuring reversible changes in axonal and myelin content and ideally should measure these changes in focal lesions and the diffuse lesions in white matter and GM. This review will consider some newer nonconventional MRI techniques, including MRS, MTI, and DTI, as tools to measure neuronal and axonal repair.

**Lesion Measures of Tissue Damage**

The initial event of lesion evolution (ie, blood-brain barrier breakdown and influx of inflammatory T cells) is documented on MRI by the presence of CELs on T1-weighted images. These active lesions persist as focal areas of hyperintense signal on T2-weighted scans, and the T2 lesion volume estimates the total burden of disease. However, T2 lesions are pathologically nonspecific and represent an entire spectrum of tissue damage, including lesions with inflammation, lesions with demyelination, gliotic scars, or lesions with severe axonal loss. Even remyelinating lesions appear hyperintense on T2 imaging sequences. Approximately 30% of CELs and T2 lesions will evolve into T1 black holes (ie, lesions with severe axonal loss and matrix destruction). This subset of hyperintense T2 lesions, which are hypointense on T1-weighted scans, is of particular interest because the axonal loss in these lesions is most likely responsible for clinical disability. In fact, T1 lesion volume correlates more closely with clinical disability than other lesion measures.

Because T1 black holes are end-stage lesions, they are useful estimates of irreversible tissue damage but will not be useful as measures of tissue repair. Nevertheless, the evolution of CELs into T1 black holes has been used to measure neuroprotection. In a large multicenter, placebo-controlled trial, Filippi et al demonstrated a neuroprotective effect of glatiramer acetate by showing a significant reduction in the percentage of CELs evolving into T1 black holes.² A large sample (1276 lesions) was analyzed in this trial, making it a labor-intensive process. T1 black hole evolution is also subject to a high degree of interobserver variability and does not account for disease in the NAWM or GM.

**MRS**

Proton MRS is the most specific measure of axonal damage because it quantitates the neuronal-specific metabolite, N-acetyl aspartate (NAA), and expresses it as a ratio to the stable brain metabolite creatine (Cr).³ A reduction in NAA/Cr ratio has been demonstrated in lesions and in NAWM and GM of patients with MS compared to healthy control subjects (Figure 1).⁴ The decrease in NAA is progressive with disease duration and correlates with cerebral atrophy, although the rate of decrease in NAA (5%/year) is higher in patients with RRMS than in those with SPMS.⁵ A robust correlation between lesion and NAWM NAA and clinical disability also has been observed. Because the reduction in NAA is partially reversible, this measure probably

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Figure 1. MRS Measures Axonal Loss in NAWM

Left panel, normal brain with measure of NAA from the voxel indicated; Right panel, MS brain with measure of NAA from NAWM (upper voxel) and from MS lesion (lower voxel).

MRS = magnetic resonance spectroscopy; MS = multiple sclerosis; NAA = N-acetyl aspartate; NAWM = normal-appearing white matter.

reflects irreversible axonal damage and a reversible axonal dysfunction. Thus MRS could potentially be used as a measure of neuroprotection and neuronal repair.

Proton MRS has been used in several small studies to demonstrate axonal recovery in patients with MS treated with disease-modifying therapies. In a study of 10 patients with RRMS treated with high-dose interferon (IFN) β-1b, Narayanan et al demonstrated an 11% increase in NAA over a 12-month period of time, using a volume of interest (VOI) measure centered over the corpus callosum. Other studies in patients with RRMS using a lower-dose IFN, shorter period of observation, or patients with RRMS with a longer disease duration have not been able to confirm these findings. However, a recent study with glatiramer acetate also showed 10% increase in NAA in the VOI over a 2-year period of time in 18 glatiramer acetate-treated patients with RRMS compared to 4 untreated patients. The interpretation of this study, however, is confounded by the significantly higher T2 lesion load in the untreated patients at entry to the study. Although MRS as a measure of neuroprotection and repair is promising, further clinical trials with larger patient groups will be necessary to assess its use.

**Cerebral Atrophy**

Cerebral atrophy is a sensitive, accurate, and highly reproducible measure of irreversible brain parenchymal tissue loss, well suited to multicenter trials with neuroprotective agents. Because 75% of brain tissue is composed of axons and myelin, a decrease in brain volume most likely reflects irreversible neuroaxonal degeneration. In fact, a strong correlation between axonal density, NAA, and cerebral atrophy has been confirmed histopathologically. The rate of atrophy in RRMS is generally estimated at 0.5% to 1% per year, approximately 10-fold higher than in age-matched healthy control subjects. Atrophy is progressive with the course of the disease and correlates with clinical disability at all time points.

Atrophy can be used as a global measure of tissue damage or regional measure of axonal loss, if segmentation techniques are used to isolate deep GM structures or the cortical ribbon. Although brain atrophy measures have been used in several clinical trials to demonstrate a therapeutic effect in slowing atrophy rate, this will most likely not be a useful technique for demonstrating tissue repair because it represents irreversible change.

**MTI**

Magnetization transfer imaging is an indirect measure of tissue damage that reflects primarily myelin loss but also correlates with axonal density on histopathologic specimens. The technique is based on the principle that protons bound to macromolecular structures (eg, myelin) are in equilibrium with the free protons responsible for the signal intensity of the image. If a radiofrequency pulse is used to selectively saturate myelin-bound protons, the signal intensity of the image decreases. The ratio of the signal intensity of images obtained with and without the saturation pulse is expressed as the magnetization transfer ratio (MTR; MTR = T1W-MT/T1W). Myelinated white matter has a relatively high MTR (0.4), GM has a lower MTR (0.32), and MS lesions have a spectrum of MTR values depending on the extent of demyelination. T1 black holes with severe axonal loss and tissue destruction have very low MTR values—some approach pure fluid values (MTR = 0) that are similar to cerebrospinal fluid. Thus, MTR can stratify T2 lesions based on myelin and axonal content. Increases and decreases in myelin content can be observed over time. Demyelination has been demonstrated by MTR in animal models of experimental allergic encephalomyelitis and toxic demyelination and in Wallerian degeneration of the feline visual system. Remyelination in MS lesions causes an increase in MTR values, a finding that has been confirmed on postmortem specimens. Surprisingly, 84% of MS lesions undergo remyelination to some extent.

Magnetization transfer ratio can be used as a global measurement of tissue destruction and/or to follow the evolution of individual CELs over time. Lesion MTR measurements confirm the heterogeneity of MS lesions. MTR values generally decrease dramatically at the time of contrast enhancement and MTR recovery (remyelination) occurs in some but not all lesions over the next 12 months. To evaluate the effect of treatment on lesion recovery, we examined 225 CELs from patients with RRMS over 24 months during a baseline versus treatment trial with IFNβ-1b. Compared to untreated baseline lesions, CELs that occurred during IFN treatment, or those that occurred during a clinical relapse requiring methylprednisolone (1 g/day for 5 days), showed significantly higher MTR values at all time points after the initial enhancement, indicating improved recovery (Figure 2). Because MTR reflects damage and repair, this imag-
ing technique will be useful for evaluating therapies aimed at neuroprotection and repair.

Global measurements of whole-brain MTR also can be obtained if MTR values for each brain voxel are calculated and plotted as a histogram. In patients with MS, the accumulation of voxels with abnormally low MTR values causes a shift in the histogram metrics compared to healthy controls. Whole-brain histograms in patients with MS have a decrease in peak height, a leftward shift of the histogram, and a lower mean (average) histogram compared to healthy control subjects. The limitation of this method of analysis is that it is relatively insensitive to change over a short period of time. Compared to lesion analysis, whole-brain MTR histogram analysis for neuroprotection trials will require a large number of patients over a long period of time to produce statistically significant data.

**DTI**

Like MTR, DTI is an indirect measure of tissue integrity. Diffusion imaging measures the random motion of protons in tissue. In MS lesions and in NAWM, the average diffusion coefficient (ADC) is increased because of the loss of structural barriers. The directionality of diffusion, expressed as fractional anisotropy, is decreased in MS because of the loss of highly aligned structures (eg, axons) or because of gliotic scar tissue. Although there are no histopathologic correlates of diffusion measurements, ADC correlates well with MTR measures and with T1 black hole hypointensity. Serial monthly diffusion measurements of CELs in 5 patients with MS were obtained over a period of 12 months.19 Interestingly, in the area of the lesions, prelesional increases in ADC were detected 6 months prior to gadolinium enhancement. Similar prelesional changes have been noted using MTR and MRS. This suggests that nonconventional MRI is more sensitive than cMRI in detecting early changes in tissue architecture. Moreover, these results may suggest that gadolinium enhancement may not indicate the inciting event in lesion formation.

**CONCLUSIONS**

Imaging of axonal damage is critical for assessing disease progression and treatment efficacy of neuroprotective agents. cMRI techniques using T1- or T2-weighted sequences (with the exception of T1 black holes) lack the specificity for neuronal axonal damage but can evaluate whether inflammation is halted. MRS is the most specific measure of axonal damage but may be difficult to implement in multicenter clinical trials. Cerebral atrophy is currently considered the best in vivo marker of axonal damage and a useful measure of neuroprotection. Because MTR can measure reversible changes in myelin content, repair strategies would need to include a measure such as lesion MTR. DTI is a promising new technique that will improve MRI correlation with clinical deficits.

**DISCUSSION**

*Dr Bar-Or:* I was curious about a couple of the slides in which you showed the serial studies with MTR and the suggestion that the MTR dropped significantly before the time point of seeing the gadolinium enhancement. Can you comment on that? Is that, do you think, just a relative insensitivity of picking up the gadolinium abnormality, or an abnormality that might be initiated at the myelin level before gadolinium enhancement?
**Dr Richert:** That is a fascinating question. I did not really dwell on it, but before the enhancement, up to 12 to 24 months beforehand, we see very small changes in MTR in the region of the lesion, if we go backwards in time rather than forwards in time. The changes are very small, maybe 5% or so, but they are definitely there. What this represents, whether this is an event that is occurring that we are not able to detect on cMRI, or whether this is purely edema, we are not really certain. I think it would be fascinating to go back even further. We have 10 years of MTR data at this point.

Now, I do not think it represents lesions that we would see, for example, with triple-dose contrast. And it would not represent lesions that we would see with delayed scanning, because we are now doing delayed scanning routinely with a 20-minute delay, and we are not seeing it there. Thus I do not think that it is just a matter of not being able to detect an enhancing lesion. I think there are really changes there. We also see them with MRS. You see very subtle changes in the area where lesions will ultimately develop. Therefore, there clearly are events before gadolinium enhancement that we just are only beginning to detect.

**REFERENCES**