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Longitudinal MR spectroscopy of neurodegeneration in multiple sclerosis with diffusion of the intra-axonal constituent *N*-acetylaspartate

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ABSTRACT

Multiple sclerosis (MS) is a pathologically complex CNS disease: inflammation, demyelination, and neuroaxonal degeneration occur concurrently and may depend on one another. Current therapies are aimed at the immune-mediated, inflammatory destruction of myelin, whereas axonal degeneration is ongoing and not specifically targeted. Diffusion-weighted magnetic resonance spectroscopy can measure the diffusivity of metabolites in vivo, such as the axonal/neuronal constituent *N*-acetylaspartate, allowing compartment-specific assessment of disease-related changes. Previously, we found significantly lower *N*-acetylaspartate diffusivity in people with MS compared to healthy controls (Wood et al., 2012) suggesting that this technique can measure axonal degeneration and could be useful in developing neuroprotective agents. In this longitudinal study, we found that *N*-acetylaspartate diffusivity decreased by 8.3% ($p < 0.05$) over 6 months in participants who were experiencing clinical or MRI evidence of inflammatory activity (n = 13), whereas there was no significant change in N-acetylaspartate diffusivity in the context of clinical and radiological stability (n = 6). As *N*-acetylaspartate diffusivity measurements are thought to more specifically reflect the intra-axonal space, these data suggest that *N*-acetylaspartate diffusivity can report on axonal health on the background of multiple pathological processes in MS, both cross-sectionally and longitudinally.

1. Introduction

Axonal degeneration is an important direct cause of permanent disability in multiple sclerosis (MS) (Trapp et al., 1998), making its detection and measurement important for monitoring disease progression and therapeutic interventions. Indeed, whereas most people with MS initially experience a disease course characterized by episodic inflammatory relapses followed by extended periods of remission toward baseline function, many eventually suffer an underlying, gradual progression of neurological deficits in motor, sensory, and cognitive function. Even with the advent of disease-modifying therapies that decrease the incidence of inflammatory episodes, neurological function eventually declines in most cases (Kappos et al., 2010).

The pathophysiological basis of this decline in neurological function remains uncertain. However, examination of spinal cord tissue obtained at autopsy from MS patients has demonstrated progressive axonal neurofilament alterations, consistent with metabolic abnormalities, in chronic lesions with very low microglia/macrophage activity (Schirmer et al., 2011). Additionally, it has been found that neurodegeneration, assessed with magnetic resonance (MR) spectroscopy and brain volume measurements, may take place in the earliest stages of MS, in a “radiologically isolated” period before the onset of clinical signs and symptoms (Kappos et al., 2010; Stromillo et al., 2013), as well as in a “clinically isolated” period before people meet full diagnostic criteria for the disease (Bergsland et al., 2012; Schirmer et al., 2011; Wattjes et al., 2007).

Abbreviations: MS, multiple sclerosis; DW-MRS, diffusion-weighted magnetic resonance spectroscopy; NAA, *N*-acetylaspartate; WM, white matter; HV, healthy volunteer; EDSS, Expanded Disability Scale Score; PASAT, Paced Auditory Symbol Addition Test; VOI, volume of interest; ICV, intracranial volume; T, Tesla

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In vivo MR techniques for measuring neurodegeneration in MS have mostly focused on the end result: atrophy, which can be measured in both brain and spinal cord on the time scale of years (Bermel and Bakshi, 2006; Fisher et al., 2008; Jones et al., 2013). These measurements demonstrate that the rate of atrophy is accelerated in MS (De Stefano et al., 2010) and that diminished brain volume measurements are correlated with clinical impairment (Gao et al., 2014; Rudick et al., 2009). Cross-sectional proton MR spectroscopy (^{1}H MRS) studies in MS have demonstrated low concentrations of *N*-acetylaspartate (NAA), an in vivo neuroaxonal marker, within lesions, in extralesional "normal-appearing" white matter (WM) (Choi et al., 2007), and the whole brain, suggesting axonal loss (Ciccarelli et al., 2007; Davie et al., 1997; Filippi et al., 2003; Ge et al., 2004; Gonen et al., 2000; Inglese et al., 2003; Kirov et al., 2009; Leary et al., 1999; Narayana et al., 2004; Oh et al., 2004; Pelletier et al., 2003; Rovaris et al., 2005; Suhy et al., 2000; Tiberio et al., 2006). However, longitudinal studies in normal-appearing WM have not been able to detect declining NAA concentration in patients with MS over a 2–3 year span (Kirov et al., 2012; Sajja et al., 2008; Tiberio et al., 2006).

Diffusion tensor imaging (DTI) has been heavily applied in MS and animal models of MS, with conflicting results. Most DTI studies in humans in vivo have demonstrated increased water diffusivity and decreased water fractional anisotropy (FA) in both lesions and normal-appearing WM (Bammer et al., 2000; Evangelou et al., 2000; Reich et al., 2010; Roosendaal et al., 2009; Werring et al., 1999). Fink et al. compared DTI measures of regions derived from tractography and brain volume analysis to distinguish the types of information contributed by these techniques (Fink et al., 2010). Their analysis showed that whereas both volumes and DTI values, such as FA, are loosely associated with composite measures of disease progression, such as lesion load and the Expanded Disability Status Scale (EDSS), they are not specific for the underlying biological process, e.g. inflammation or neurodegeneration (Samann et al., 2012).

In a previous cross-sectional study, we measured diffusion of NAA in the human normal-appearing corpus callosum on a 7-Tesla (T) MRI scanner, comparing 15 people with MS and 14 healthy controls (Wood et al., 2012). The corpus callosum is a good location for studying axonal degeneration from anatomical, functional, and technical standpoints: it is the largest WM structure; it is involved in multiple sensory, attentional, and cognitive processes; and the fibers are quasi-coherent in orientation. We found that NAA *parallel diffusivity* (λ), i.e. diffusivity along the axonal propagation direction, was on average significantly diminished in MS and, importantly, inversely correlated with both DTI measures, i.e. mean diffusivity (MD) and fractional anisotropy (FA), and with clinical severity (EDSS). Our findings provided preliminary evidence that diffusion-weighted MR spectroscopy (DW-MRS) of NAA can distinguish axonopathy from other processes such as inflammation, vasogenic edema, demyelination, and gliosis. Through subsequent studies with healthy volunteers, we demonstrated that axonal modeling can be used to yield NAA diffusivity values that more accurately reflect the cytosolic diffusion coefficient of NAA while minimizing the confounding effects of inter-subject differences and large spectroscopy voxels (Ronen et al., 2014).

In MS, axonal degeneration plays out over time. In this study, therefore, we asked whether the DW-MRS technique is capable of detecting axonopathy in WM tissue over a period of six months. For this, we evaluated a new cohort of MS patients that was divided into groups with stable and active disease courses, and a third group of healthy controls allowed us to add cross-sectional data at the two time points. In the process, we also established that DW-MRS is feasible in a clinical setting with a 3 T scanner.

2. Materials and methods

2.1. Participants

The National Institutes of Health Institutional Review Board approved this study. All participants gave informed consent. Participants were neurologically evaluated in the Neuroimmunology Clinic, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, Maryland, USA.

The MS cohort was recruited from the Neuroimmunology Clinic and consisted of a wide range of cases – from stable cases with no new lesions for 1–10 years (based on existing prior MRI scans) to cases with high disease activity where new T_2 lesions had formed in the 6 months before or during the study. For this study, 19 participants who met McDonald criteria for MS (Polman et al., 2011) were scanned. At recruitment, 9 were stable – they had not had a relapse or new T_2 lesion for at least 1 year. Three of these had a new T_2 lesion during the study so were reclassified as active. Therefore, at study conclusion, there were 6 stable cases and 13 active cases. Three stable and 10 active cases were on disease-modifying therapies.

Healthy volunteers (HVs) were recruited from the NIH Clinical Research Volunteer Program. The HVs had no history of neurological conditions. Each HV was examined by a neurologist and underwent clinical MRI scans that were within normal limits. HVs were compensated for taking part in the study.

All MS cases were scanned at baseline ("month 0") and month 6. Most active MS cases were also scanned at month 3 in order to more closely follow disease activity. Five of the 6 HVs were scanned twice, with scans separated by 1–14 days. For all repeat scans, volumes of interest were positioned to match, as much as possible, the original placement.

MS cases were assessed at month 0 and month 6 with the EDSS, Paced Auditory Symbol Addition Test (PASAT, 3-second version), 9-Hole Peg Test, and 25-Foot Timed Walk. By design, clinical data were obtained within 30 days of MRI acquisition. The average time lag between MRI and clinical exam was 2 days for both stable and active patients, with 32 of 38 scans obtained within 1 day of clinical exam and the remaining occurring within 6, 14, 27, 28, 30 and 30 days of the clinical exam.

During each scan session, structural and DW-MRS scans were acquired for all participants. All scans were acquired on 3 T Philips Achieva scanners (Philips Medical Systems, Best, The Netherlands) in the NIH Clinical Center Radiology and Imaging Sciences Department. These scanners have gradients with a maximum amplitude of 80 mT/m and a slew rate of 100 T/m/s, quadrature volume transmit coils, and 8-channel receive head coils.

2.2. Structural image acquisition and processing

All subjects were scanned with the following order of sequences: 3D T_1-weighted gradient echo, DTI, two DW-MRS volumes of interest (VOIs), and a T_2-weighted FLAIR. Lastly, gadolinium-enhanced T_1-weighted scans (see below) were acquired for all active MS cases and for stable cases at the discretion of the clinician.

3D T_1-weighted gradient echo images were acquired with an inversion-prepared turbo field echo (TFE) sequence and were used for positioning of the VOI in the DW-MRS experiments and for tissue segmentation in the post-processing stage. Imaging parameters were: field of view (anterior-posterior × foot-head × right-left) = 240 × 240 × 180 mm^3, 1 mm isotropic resolution, TR/TE = 7.00 ms/3.15 ms, TI = 874.2 ms, SENSE = 2(AP) × 3(RL), and total scan time = 5.30 min + delay for scanner preparation. Whole brain DTI images were acquired using single-shot 2D spin-echo echo-planar imaging. DTI parameters were: field of view = 224 × 224 × 120 mm^3, 2 × 2 × 2 mm^3 isotropic resolution, TR/TE = 7487 ms/85 ms, 32 diffusion weighting directions with b = 800 s/mm^2, SENSE = 3(AP), and total scan time = 5.50 min.

T_2-weighted Fluid Attenuated Inversion Recovery (FLAIR) images were acquired for all patient scan sessions using a 3D–FLAIR-VISTA (volume isotropic turbo spin-echo acquisition) sequence with parameters: field of view = 240 × 240 × 180 mm^3, 1 mm isotropic resolution, TR/TE = 4800 ms/365 ms, TI = 1600 ms, SENSE = 2.6(AP) × 2(RL), and