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^{1}H-MRSI evidence for cortical gray matter pathology that is independent of cerebral white matter lesion load in patients with secondary progressive multiple sclerosis

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ABSTRACT

We examined: *(i)* neuro-axonal disturbance (as indicated by ^{1}H-MRSI NA/Cr values) in the cortical grey matter (*cGM*) of 10 untreated patients with relapsing–remitting (*RR*) and 10 with secondary-progressive (*SP*) multiple sclerosis (*MS*), and *(ii)* the relationships between cGM-NA/Cr values and the degree of EDSS-measured clinical disability and cerebral white-matter (*WM*) lesion load (*LL*) in these patients. Whereas mean and median cGM-NA/Cr values in our RR group were similar to those in 18 age-matched normal controls (*NC*), large statistically-significant decreases (between 14.3% and 18.5%) were found in our SP group relative to both our RR and NC groups. When data from all patients was combined, we found: *(i)* a large negative correlation between EDSS scores and cGM-NA/Cr values ($r=-0.55$); and *(ii)* a larger negative correlation of cGM-NA/Cr values with cerebral T1-hypointese WM-LL (*T1-LL*, $r=-0.73$) than with cerebral T2-hyperintense-LL (*T2-LL*, $r=-0.63$). Importantly, *(i)* correlations of WM-LL with cGM-NA/Cr were larger in the RR group than in the SP group (T1-LL: $r=-0.79$ vs. -0.54; T2-LL: $r=-0.63$ vs. -0.51), and *(ii)* cerebral WM-LL values could not fully account for the extent of the decrease in mean cGM-NA/Cr that was seen in our SP group relative to our NC group. Our observations are consistent with the possibilities that: *(i)* in patients with RR-MS, ^{1}H-MRSI-measured cGM neuro-axonal disturbances are strongly related to the effects of axonal transection that are associated with cerebral WM lesions; and *(ii)* in patients with SP-MS, such cGM neuro-axonal disturbances are more severe and are associated with a more-widespread degenerative process (which probably includes a considerable degree of cortical demyelination).

1. Introduction

Pathology of the cortical gray matter (*cGM*) in patients with multiple sclerosis (*MS*) has long been recognized on detailed histopathological examination [1]. Nevertheless, MS has traditionally been considered a demyelinating disease of the white matter (*WM*) of the central nervous system because, until recently, cGM pathology has routinely been underestimated by standard techniques. For example, cGM lesions are not evident on conventional *post-mortem* examinations; [2] this is because: *(i)* cortical myelin is not readily apparent on routine histological staining with Luxol fast blue; and *(ii)* cortical lesions are not hypercellular and, therefore, are not obvious on hematoxylin-eosin-stained sections. Furthermore, the majority of cGM lesions are also not evident on conventional magnetic resonance imaging (*MRI*) examinations, [3–5] even at higher fields; [6] this is because: *(i)* cGM lesions are often either small or thin, which make them subject to partial volume effects on MRI; and *(ii)* they are associated with much less inflammation and demyelination than is typical of WM lesions [7,8] and, as a result, they are associated with very little contrast on conventional T_2-weighted or T_1-weighted MRI. Within the last decade, however, advances in the neurohistopathological and neuroimaging analysis of tissue from patients with MS have fostered a renewed appreciation for the importance of cGM pathology in this disease [9–16].

1.1. Histopathological studies of cGM lesions in patients with MS

A number of *post-mortem* and *ex-vivo* histopathological studies have now described and quantified cGM lesions in the brains of patients with MS [1,3,17,7,8,4,18–24]. The prevalence of such cGM lesions can be inferred from the results of the study by Peterson et al. [7] in which as many as 112 cortical lesions were identified by an immunocytochemical analysis in 110 tissue blocks from 50 MS patients. Importantly, in this study, cGM lesions showed evidence of: *(i)* demyelination; *(ii)* axonal and dendritic transection; and *(iii)* neuronal apoptosis, particularly in neurons whose axons showed demyelination. Furthermore, the elegant work of Kutzelnigg et al. [18] has further illustrated the prevalence of cGM pathology in MS and has suggested that cortical demyelination is much more prominent in the ultimate, progressive phase of MS than in the initial, relapsing–remitting phase of the disease.

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1.2. ^{1}H-MRS studies of neuro-axonal integrity in patients with MS

As we have reviewed elsewhere, [25] proton magnetic resonance spectroscopy (*^{1}H-MRS*) and spectroscopic imaging (*^{1}H-MRSI*) studies of patients with MS have been important in detecting and quantifying neuro-axonal injury *in vivo*. This is done based on the signal intensity of the neuronal marker compound, N-acetylaspartate (*NAA*), which is localized within neurons and neuronal processes [26,27]. Although NAA has been detected in other cells *in vitro*, particularly O2A progenitor cells, [28,29] this phenomenon does not appear to be relevant *in vivo*; indeed, complete degeneration of rat optic nerve following transection has been demonstrated to be associated with complete loss of NAA, despite the continued presence of proliferating oligodendroglial cells [30]. As a result, the amount of ^{1}H-MRS(I)-measured NAA or, most commonly, the amount of total N-acetyl (*NA*) groups that resonate at 2.0 ppm on the ^{1}H-MR spectrum (of which NAA is the major component in the adult brain), has been used as a non-invasive *in vivo* indicator of neuro-axonal integrity [25].

Whereas absolute and semi-absolute *in vivo* quantification of ^{1}H-MRS(I)-measured NA is now common practice, we and others prefer the use of the total amount of ^{1}H-MRS(I)-measured creatine and phosphocreatine (*Cr*) in the same voxel as an internal standard [25]. Importantly, such a "ratio" approach corrects for many of the sources of variability that can affect estimates of absolute concentration. For example, because Cr is present in virtually all types of brain tissue but is not present in the cerebrospinal fluid, NA/Cr ratios are not sensitive to the effects of brain atrophy and sulcal enlargement — effects that have been shown to be present even in the early, relapsing–remitting stage of MS [31] and that can potentially confound the results of absolute or semi-absolute methods of quantitation. Of course, this normalization-to-Cr approach is only reliable if the within-voxel Cr concentration being measured is unaffected by the within-voxel pathology of the tissue that is being studied; however, as our recent meta-analysis of published data suggests, this seems to be true for normal-appearing cGM in patients with MS [25].

^{1}H-MRSI studies have consistently found decreased NA/Cr values in both the lesional and normal-appearing WM of patients with MS [32–34]. Although a small number of studies have found values of ^{1}H-MRS(I)-measured NA to be statistically unaffected in the cGM of patients with MS, [35–37] the majority of such studies have provided evidence for decreased cGM NA: [38–44] and NA/Cr [45–47]. This is true for: *(i)* patients with clinically-isolated syndromes indicative of MS (*CIS*); [47] *(ii)* patients in the early, relapsing–remitting stage of the disease; [38,46,44] and *(iii)* patients with more-severe, progressive MS [40–43]. Importantly, in each of the studies that explicitly compared groups of patients with relapsing–remitting MS to those with progressive MS and found a significant decrease in cGM NA, this decrease was far greater in the group of patients with progressive MS [40,41,43]. This finding seems to be consistent with both: *(i)* the histopathological findings of Kutzelnigg et al. [18] regarding more-severe cortical demyelination in the progressive phase of MS; and *(ii)* the histopathological findings of Peterson et al. [7] which suggest that such cGM demyelination is associated with neuronal disturbance in the cortex.

1.3. cGM pathology secondary to WM lesions in patients with MS

In addition to the different types of cGM lesions that have been described histopathologically, [7] cGM that is not directly affected by macroscopic lesions in patients with MS may also be affected indirectly by neuronal and dendritic changes that are secondary to axonal injury within WM lesions [48,30]. For example, WM lesions in patients with MS are often associated with the transection of axons [48,30] and the subsequent Wallerian degeneration of the distal portion of these axons. Because many of these axons synapse on cortical dendrites and cell bodies, loss of these inputs (and the trophic factors transmitted at the synapses) can also lead to anterograde dendritic and neuronal pathology [49,50]. Furthermore, proximal axons that are still connected to cell bodies can undergo retrograde degeneration, [51] which can eventually result in the death of the neuronal cell by apoptosis [52].

1.4. The relationship of cGM pathology to cerebral WM lesion-load and clinical status in patients with MS

A number of recent studies have examined the relationship between cerebral WM lesions and cGM pathology in patients with MS. For example, in a very small study published in 2007, Bo et al. [21] compared three patients with "extensive subpial cGM demyelination" (*ESD*) to three patients with "minimal subpial cGM demyelination" and found that the ESD group had a greater percent cerebral-WM-LL (5.3% vs. 2.7%); this difference did not reach statistical significance, however, that may have been due to the low power associated with the very small sample size. With regards to the relationship between cerebral WM lesions and ^{1}H-MRS(I) measures of cGM pathology, Van Au Duong et al. [47] studied cGM-NA/Cr values and cerebral WM inflammation in patients with CIS and found a significantly-lower mean NA/Cr value in the cGM of those 15 patients who had gadolinium-enhancing cerebral WM lesions than in the 25 patients who did not have any such lesions (and whose mean cGM-NA/Cr value did not differ from that of their normal controls). On the other hand, a number of ^{1}H-MRS(I) studies of cGM neuro-axonal integrity in patients with MS have failed to find statistically-significant correlations between cerebral WM lesion-load (*WM-LL*) and cGM-NA [38,40,37,44] or between cerebral WM-LL and cGM-NA/Cr [47]. Similarly, whereas some studies have found statistically-significant correlations between cGM-NA and clinical status [42] and between cGM-NA/Cr and memory impairment in patients with MS, [46] other studies have failed to find statistically-significant correlations between cGM-NA and clinical disability in such patients [38,39,36,40,41,37]. It should be noted, however, that all of these studies had relatively-low sample sizes and some of them had a very limited range of cerebral WM-LL values [38,47] or scores on Kurtzke's Expanded Disability Status Scale [53] (*EDSS*) [38] — either of which would make it very difficult to find statistically-significant correlations. Thus, to the best of our knowledge, the relationship of ^{1}H-MRS(I) measures of cGM neuro-axonal integrity to cerebral WM-LL and clinical status in patients with MS has not yet been definitively established.

In the present study, we: *(i)* used conventional MRI to quantify cerebral WM-LL values in 10 patients with relapsing–remitting (*RR*) MS and 10 patients with secondary-progressive (*SP*) MS, *(ii)* used ^{1}H-MRSI NA/Cr values to estimate *in vivo* the degree of neuro-axonal disturbance in the cGM of these same two groups of patients relative to a group of 18 age-matched normal controls, and *(iii)* examined the relationships between our patients' mean cGM-NA/Cr values and both their EDSS scores and their cerebral WM-LL values. This was done both for our patients' cerebral WM-LL of: *(i)* hyperintensities on T_2-weighted MRI (*T2-LL*);[54] and *(ii)* hypointensities on T_1-weighted MRI (*T1-LL*), which are more specific markers of tissue destruction and axonal loss in patients with MS, and which correlate better with clinical disability than do T2-LL values [55].

2. Methods

2.1. Subjects

^{1}H-MRSI and conventional MRI data were obtained from 10 untreated patients with RR-MS (7 females, 3 males) and 10 untreated patients with SP-MS (5 females, 5 males). These were all patients who were followed in the MS Clinic at the Montreal Neurological Institute and Hospital (*MNI/H*). Levels of clinical disability in these patients were assessed using Kurtzke's EDSS [53]. Similar data were collected