Spinal Cord Atrophy in Multiple Sclerosis Caused by White Matter Volume Loss

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Objective: To assess the relative contributions of white matter (WM) and gray matter (GM) volume loss to spinal cord atrophy in multiple sclerosis (MS).

Design: Postmortem study of transverse sections obtained from 5 levels of the spinal cord, with measurement of the cross-sectional GM and WM areas.

Setting: Department of Neuropathology, University of Nottingham, Nottingham, England.

Patients: Fifty-five MS cases and 33 controls.

Main Outcome Measures: Size of the WM and GM areas.

Results: The WM area was significantly reduced in MS cases in the upper but not the lower cord levels. The GM area was not significantly different between MS and control cases.

Conclusion: Spinal cord atrophy in MS is due to WM rather than GM volume loss.

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PINAL CORD ATROPHY IN MULTIPLE sclerosis (MS) has been shown to correlate with clinical disability.1 Growing evidence suggests that tissue loss in the brain is not restricted to the white matter (WM) but also affects gray matter (GM) structures.2-3 However, the extent of GM atrophy in the spinal cord has not been adequately examined.

Characterizing the extent of GM atrophy in the brain and spinal cord is necessary to better understand mechanisms of permanent disability in MS. The resolution of current magnetic resonance imaging (MRI) equipment is not sufficiently high to study the spinal cord GM in vivo. Histopathologic studies are therefore required to investigate spinal cord GM disease. The aim of this postmortem study was to assess the relative contributions of WM and GM tissue loss to spinal cord atrophy in MS.

METHODS

CLINICAL MATERIAL

Human autopsy material was obtained from 55 pathologically confirmed cases of MS and 33 controls. This material was obtained from the Department of Neuropathology, Oxford Radcliffe NHS Trust, and comprised all of the MS and control cases available. The patients with MS (29 men and 26 women) were aged 25 to 83 years (mean age, 57.5 years), with a disease duration of 2 to 43 years (mean disease duration, 17.1 years). The controls (15 men and 18 women), aged 31 to 81 years (mean age, 57.9 years), had no clinical or pathological evidence of spinal cord disease. The local research ethics committee approved the study. This autopsy material has been used in 2 recently reported studies.4,5

PREPARATION OF THE SECTIONS

For each of the MS and control cases, formalin-fixed, paraffin-embedded transverse sections were obtained from 5 levels of the spinal cord (upper cervical, lower cervical, upper thoracic, lower thoracic, and lumbar levels). These 15-µm-thick sections were stained for neuronal elements (with Palmgren silver) and myelin (Luxol fast blue cresyl violet) using a protocol described by Lowe and Cox.6

MEASUREMENTS OF GM AND WM AREAS

A total of 384 Palmgren silver sections were digitally photographed at low power (Olympus DP10 camera [Olympus UK Ltd, Southall, England] mounted on a Leica WILD MZ8 dissecting microscope [Leica Microsystems...
The Palmgren silver sections were used to improve the blinding of the observer (C.P.G.) to the disease state because the myelin-stained sections highlight demyelination. The cross-sectional GM areas were traced manually using image analysis software (AnalySIS Pro running SIS software, Olympus UK Ltd) (Figure 1). The Palmgren silver sections, examined via microscopy (x10), were used as a reference to help identify the GM boundaries. The cross-sectional areas of the spinal cords were measured and the WM cross-sectional area calculated (ie, total spinal cord area minus the GM area). A shrinkage factor of 0.71, calculated in our laboratory in a previous study,7 was applied to the measured areas to correct for changes in tissue size as a result of fixation and embedding processes. In this way, comparisons can be made between our spinal cord areas and published MRI studies.

It was not possible to accurately identify the GM boundaries on a few sections, either because there was a tear in the section or there was gross disruption of the normal tissue architecture secondary to an MS plaque. Measurements were obtained from 356 of the 384 Palmgren silver sections. It was possible to obtain measurements from an additional 12 sections using the myelin-stained material. In 16 cases, it was possible to measure only the GM area on half of the section; in these cases, this area was doubled to give an estimate of the GM area. It was not possible to obtain measurements of the remaining 16 sections because the GM boundaries could not be accurately identified.

VALIDATION AND REPRODUCIBILITY OF METHODS

To evaluate intraobserver reproducibility, the GM areas of 45 randomly selected Palmgren silver sections were measured on 2 separate occasions (coefficient of variation, 1.36%). To validate the use of the myelin-stained sections for some of the measurements, the GM areas for 15 Palmgren silver sections and the corresponding 15 Luxol fast blue sections were measured (coefficient of variation, 2.94%). Fifteen Luxol fast blue sections were measured on 2 separate occasions (coefficient of variation, 1.42%).

STATISTICAL ANALYSIS

Multiple regression analyses were used to examine the influence of age, sex, cord location, disease state, and in MS cases, disease duration on cross-sectional GM and WM areas. The regression coefficient was calculated for each of these variables. We do not have detailed clinical information regarding disability and are therefore unable to examine the correlation between clinical disability (Expanded Disability Status Scale) and tissue atrophy.

RESULTS

WM AREA MEASUREMENTS

The cross-sectional WM area of the spinal cord was significantly reduced in the MS cases compared with controls, controlling for age, sex, and cord location (regression coefficient, −5.80; P = .001; ie, controlling for other variables, the WM area was reduced in the MS cases by an average of 5.80 mm²). Specifically, the WM area was reduced at the upper cervical (regression coefficient, −12.17; P = .04), lower cervical (regression coefficient, −10.47; P = .04), and upper thoracic (regression coefficient, −7.50; P = .02) levels in MS cases but not at the lower thoracic (regression coefficient, 0.55; P = .84) or lumbar (regression coefficient, −1.55; P = .60) levels (Figure 2A). As expected from previous work,3 the WM area in the MS cases was strongly influenced by the disease duration (regression coefficient, −0.71; P = .001).

GM AREA MEASUREMENTS

In contrast to the WM measurements, the cross-sectional GM area was not significantly different between MS cases and controls (regression coefficient, −0.15; P = .70). Similarly, the GM area was not reduced at the upper cervical (regression coefficient, −0.26; P = .75), lower cervical (regression coefficient, −0.27; P = .73), upper thoracic (regression coefficient, −0.04; P = .95), lower thoracic (regression coefficient, 0.26; P = .59), or lumbar (regression coefficient, −0.51; P = .74) levels in MS cases (Figure 2B). The GM area in the MS cases was not significantly influenced by the disease duration (regression coefficient, 0.03; P = .13).

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COMMENT

To our knowledge, our series represents the largest postmortem study of spinal cord atrophy in MS. We demonstrate that spinal cord atrophy in MS is virtually exclusively due to WM volume loss. Although we do not have detailed retrospective information regarding disability, our results suggest that previously observed correlations between cord atrophy and disability reflect WM rather than GM volume loss.

Our finding of WM atrophy restricted to the upper regions of the spinal cord is consistent with previous work using this postmortem material. These studies have demonstrated that whole-cord atrophy and atrophy of the lateral and posterior WM columns are not observed in the lower cord.

Although the cervical cord has a predilection for demyelinating WM lesions, tissue loss within WM lesions does not have a significant influence on local cord atrophy. However, the distribution of WM lesions within the cord may still influence the pattern of atrophy through the effects of Wallerian degeneration. For example, the preservation of ascending WM tracts in the lower cord may reflect the paucity of WM lesions in this region and explain, in part, the normal WM volume in the lower cord.

Only one other study, to our knowledge, has reported the relative contributions of GM and WM atrophy in the spinal cord. Bjartmar et al. studied spinal cord disease in postmortem spinal cord specimens from severely disabled patients with MS and 6 controls, demonstrating a significant reduction in the spinal cord cross-sectional area in the MS cases. The GM-WM ratio in the MS cords was similar to controls, suggesting that atrophy affects both GM and WM.

Our results suggest that the spinal cord GM volume is well preserved in MS. In contrast, MRI studies of the brain demonstrate reductions in cortical thickness and thalamic volume in patients with MS. Similarly, in a postmortem study, Cifelli et al. reported a mean 21% reduction in the volume of the medial dorsal nucleus of the thalamus in MS cases compared with controls. Loss of neuronal tissue is likely to contribute to the substrate of this GM atrophy in the brain, as suggested by magnetic resonance spectroscopy studies demonstrating reductions in N-acetylaspartate levels in the cerebral cortex and thalamus.

Although the mechanisms of GM atrophy in the brain are poorly understood, one possibility is a direct effect of local GM demyelinating lesions. It remains unknown whether the reductions in neuronal size and number observed in the thalamic GM in MS are related to the presence of such lesions. Extensive GM lesions, as described in the cerebral cortex, could contribute to GM atrophy via myelin loss, neurite transection, or apoptotic neuronal death. It is possible that the extent or pattern of GM demyelination is influenced by location, leading to regional variations in GM atrophy. For example, a distinct pattern of subpial demyelination accounts for a high proportion of the total cortical demyelinated area.

Alternatively, GM atrophy may occur as a consequence of distant WM lesions. Axonal transections in such lesions may result in neuronal disease through retrograde or anterograde transynaptic degeneration. It has been suggested that the extensive connections of the thalamus with other brain structures make it particularly sensitive to the effects of disease in distant sites. In comparison, the spinal cord GM may be less susceptible to the effects of distant WM lesions owing to differences in its connectivity.

There are limitations to our study. In some of the MS cases, disruption of the tissue architecture was observed on the Palmgren silver sections, potentially interfering with the blinding of the study. The use of a small number of myelin-stained sections may also have interfered with blinding. Despite our efforts to measure the GM area on each of the sections, this was not possible in every case, particularly when the GM boundaries were indistinct owing to local MS plaques. This may have biased the sample to include a greater proportion of “normal-appearing” material. However, given the large number of sections included in our study, we are confident that these factors have not significantly influenced our results.

Our results suggest that spinal cord atrophy in MS is purely due to WM volume loss. Our observation of preserved GM volume is a notable one, highlighting important differences between the GM of the spinal cord and the GM structures of the brain. A greater understanding of the mechanisms of GM disease is required to explain these differences.
REFERENCES


