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MRI quantification of gray and white matter damage in patients with early-onset multiple sclerosis

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Abstract *Background and objective* Contrary to what happens in adult-onset multiple sclerosis (MS), in a previous preliminary magnetic resonance imaging (MRI) study we showed only subtle normal-appearing brain tissue changes in patients with early-onset MS. Our objective was to evaluate the presence and extent of tissue damage in the brain normal-appearing white matter (NAWM) and gray matter (GM) from a larger population of patients with early-onset MS. *Methods* Using diffusion tensor (DT) and magnetization transfer (MT) MRI, we obtained DT and MT ratio (MTR) maps of the NAWM and GM from 23 patients with early-onset MS and 16 sex- and age-matched healthy volunteers. *Results* Compared with healthy volunteers, patients with early-onset MS had significantly increased average MD ($p = 0.007$) and FA peak height ($p = 0.007$) and decreased average FA ($p < 0.0001$) of the NAWM. Brain dual-echo lesion load was significantly correlated with average FA ($r = -0.48$, $p = 0.02$) and with FA peak height ($r = 0.45$, $p = 0.03$) of the NAWM. No MTR and diffusion changes were detected in the GM. *Conclusions* This study confirms the paucity of the 'occult' brain tissue damage in patients with early-onset MS. It also suggests that in these patients GM is spared by the disease process and that NAWM changes are likely to be secondary to Wallerian degeneration of fibers passing through macroscopic lesions.

Key words magnetic resonance imaging · multiple sclerosis · diffusion tensor MRI · magnetization transfer MRI

Introduction

In adult-onset multiple sclerosis (MS), postmortem studies have shown subtle and widespread abnormalities in the normal-appearing white matter (NAWM), which include diffuse astrocytic hyperplasia, patchy edema, and perivascular cellular infiltration, as well as axonal damage [1–3]. More recently, it has also been shown that a considerable proportion of MS pathology is located in the cerebral gray matter (GM) [4–6]. In all the adult-onset MS phenotypes, modern magnetic resonance (MR) techniques, including magnetization transfer (MT) and diffusion tensor (DT) MRI, have confirmed: a) the presence of diffuse NAWM changes, which are only modestly correlated with the amount of focal T2-weighted lesions [7, 8]; b) that GM damage is an important component of the disease [7, 8], which is progressive since its relapsing-remitting (RR) phase onward [9]; and c) that 'occult' damage to the NAWM and GM contributes to the development of brain atrophy [7].

Although MS can occur before 16 years of age in about 3–12 % of cases [10], only a preliminary study has attempted to quantify the extent of brain and the cervi-

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cal cord damage in these patients, using imaging (MRI) [11]. This study showed that, contrary to what happens in adult-onset MS, patients with early-onset MS only have mild damage to the normal-appearing brain tissue [11] and suggested that this could be a factor to explain the more favorable course of this form of MS compared with the 'classical' one. This study, however, left three questions unanswered: a) Are NAWM and GM equally involved in early-onset MS?; b) Does this damage result in irreversible tissue loss (brain atrophy)? and c) Which is the relationship between focal macroscopic lesions and diffuse NAWM changes in these patients? This study was undertaken to respond to these questions with the ultimate goal of improving our understanding of the pathophysiology of early-onset MS.

Patients and methods

Patients

We studied 23 patients with early-onset MS (15 girls and 8 boys; mean age = 14.1 years, range = 7–16 years; mean disease duration = 28 months, range = 8–56 months; median Expanded Disability Status Scale score (12) = 1.0, range = 0.0–6.5; and mean number of relapses per patient = 2.7, range = 2–5). Sixteen healthy volunteers (11 girls and 5 boys; mean age = 14.7 years, range, 9–16 years) served as control subjects. At the time MRI was performed, all patients had been relapse- and steroid-free for at least six months. Fourteen of them were taking disease-modifying treatments (interferon beta-1a = 9 patients, glatiramer acetate = 3 patients and mitoxatrone = 2 patients). Local Ethical Committee approval and written informed consent from each subject were obtained prior to study initiation.

MRI acquisition

Using a 1.5 T scanner, the following sequences of the brain were obtained from all subjects: a) dual-echo turbo spin-echo (TSE) (TR/TE = 3300/16-8; 24 axial 5 mm-thick slices with 256×256 matrix, and 250×250 mm^2 FOV); b) 2D gradient echo (GE) (TR/TE = 600/12, $\alpha = 20°$, 20 axial 5 mm-thick slices with 256×256 matrix, and 250×250 mm^2 FOV) with and without a saturation pulse (off-resonance RF pulse centered 1.5 kHz below the water frequency, with a Gaussian envelope of duration of 7.68 ms and $\alpha = 500°$); c) pulsed gradient spin echo (PGSE) echo planar (interecho spacing = 0.8, TE = 123; 10 axial 5 mm-thick slices with 128×128 matrix, and 250×250 mm^2 FOV), with diffusion gradients applied in eight non-collinear directions. Additional information about this sequence is given elsewhere [13]. d) T1-weighted SE (TR/TE = 768/15, 24 axial 5 mm-thick slices with 256×256 matrix, and 250×250 mm^2 FOV).

For dual echo, T1-weighted and GE scans, the slices were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum. For the PGSE scans, the same orientation as in the other scans was used, with the second-last caudal slice positioned to match exactly the central slices of the other image sets. This brain portion was chosen as these central slices are less affected by the distortions due to B_0 field inhomogeneity, which can affect image co-registration.

MRI postprocessing

All MRI postprocessing was performed by a single observer blinded to subjects' identity. Brain lesions were identified on the dual-echo scans and lesion volumes were measured using a segmentation technique based on a local thresholding [14]. After co-registration of the two GE scans using a surface-matching technique based on mutual information, MT ratio (MTR) images were derived pixel-by-pixel, as described elsewhere [13]. Extra-cerebral tissue was removed from MTR maps using a local thresholding segmentation technique [14], and the resulting images were co-registered with the T2-weighted images. PGSE images were first corrected for distortion induced by eddy currents using an algorithm which maximizes mutual information between the diffusion un-weighted and weighted images [15]. Then, the diffusion tensor was calculated, and mean diffusivity (MD) and fractional anisotropy (FA) derived for every pixel, as previously described [13]. The diffusion images were interpolated to the same image matrix size as the dual-echo, and then the b = 0 step of the PGSE scans were co-registered with the dual-echo T2-weighted images [15]. Using SPM99 and maximum image in-homogeneity correction [16], brain GM, WM, and cerebrospinal fluid (CSF) were automatically segmented from dual-echo images. Each pixel was classified as GM, WM or CSF, dependent on which mask had the greatest probability (maximum likelihood) at that location. This generated mutually exclusive masks for each tissue. The resulting masks were superimposed onto the MTR, MD and FA maps (on which hyperintense lesions were masked out previously), and the corresponding MTR, MD and FA histograms of the NAWM and GM were produced. FA histograms were derived only for the NAWM, since no preferential direction of water molecular motion is expected to occur in the GM, due to the absence of a microstructural anisotropic organization of this tissue compartment. For each histogram, the average MTR, MD and FA and the peak heights were measured. Given the strong correlation existing between average histogram measures and the histogram peak location, the latter quantity was not considered for this study, to reduce the risk of type I errors.

T1-weighted images were used to measure the normalized brain volumes (NBV), using the cross-sectional version of the Structural Imaging Evaluation of Normalized Atrophy (SIENAx) software [17]. SIENAx uses a brain extraction tool, to perform segmentation of brain from non-brain tissue in the head and to estimate the skull surface. Then, the extracted brain image is segmented into WM, GM and CSF, yielding an estimate of the absolute volumes of brain tissue compartments. The original MRI image is registered to a canonical image in a standardized space (derived from the MNI152 standard space) to provide a spatial normalization scaling factor for each patient. The estimated absolute volumes for each subject are then multiplied by the normalization factor, to yield a normalized parenchymal volume of these tissue compartments.

Statistical analysis

A two-tailed Student's t-test for non-paired data was used to compare MTR, MD and FA histogram-derived metrics and NBVs between patients and controls. Univariate correlations were explored using the Spearman rank correlation coefficient.

Results

No T2-weighted abnormalities were seen in any of the normal volunteers. In early-onset MS patients, mean brain T2-weighted lesion volume was 13.1 ml (range = 1.0–61.5 ml), average lesion MTR was 38.1 % (SD = 2.7 %), average lesion MD was 1.20×10^{-3} mm^2/s (SD = 0.08×10^{-3} mm^2/s), and average lesion FA was 0.26 (SD = 0.03). NBV was 1125 ml (SD = 68 ml) in early-onset MS patients and 1144 ml (SD = 137 ml) in controls (p = n. s.). No difference between the two groups was de-

tected when comparing the WM and GM volumes, separately (data not shown).

In the Table, MTR, MD and FA histogram derived metrics of the brain NAWM and GM from early-onset MS patients and controls are reported. Compared to healthy volunteers, patients had significantly increased average MD ($p = 0.02$) and FA peak height ($p = 0.007$) and decreased average FA ($p < 0.0001$) of the NAWM. No MTR and diffusion changes were detected in the GM.

Brain dual-echo lesion load was significantly correlated with average FA ($r = -0.48$, $p = 0.02$) and with FA peak height ($r = 0.45$, $p = 0.03$) of the NAWM.

Discussion

The main result of this study is the demonstration that brain GM is spared by the pathological process in patients with early-onset RRMS, as indicated by the preserved volume and the absence of any MTR and diffusion abnormalities of this brain compartment. This contrasts with findings in adult-onset MS, where variable extents of GM volume loss [18, 20] and intrinsic damage to the remaining tissue [13, 21–25] have been consistently shown virtually in all MS patients with different disease courses. GM loss and intrinsic damage have indeed been found not only in patients with the progressive forms of the disease [18, 21, 23], but also in those with early RRMS [20, 24] as well as in those with a single clinical episode suggestive of MS [25]. In addition, recent longitudinal studies have shown that GM damage evolves over time in RRMS [9, 26, 27] and progressive MS [28, 29] patients and that it might develop faster than WM damage at the beginning of the disease [26, 30]. In patients with adult-onset MS, recent work has shown a moderate correlation between GM damage and the clinical manifestations of the disease [31], as well as that GM damage is an independent predictor of the accumulation of disability over a five-year follow up [32]. These data coupled with those of the present study thus suggest that GM sparing in patients with early-onset MS might yet be another factor, in addition to the low rate of development of hypointense lesions and brain atrophy [33] and the absence of "occult" cord pathology [11], to explain why the disease course of such patients is on average more favorable than that of patients with adult-onset MS [10, 34]. Clearly, we can not completely exclude that the application of other MR techniques, such as proton MR spectroscopy, might have disclosed subtle GM damage in our patients. Nevertheless, three different MR techniques showed no GM abnormalities in our patient sample, thus making this possibility unlikely.

The second main result of the present study is the demonstration that diffuse NAWM pathology does occur in patients with early-onset MS, albeit at a lesser degree than in patients with adult-onset MS [13, 21–23]. This confirms and extends previous findings from our group showing subtle abnormalities of the normal-appearing brain tissue on a smaller cohort of these patients [11]. With this in mind, a major issue to be addressed is to attempt to define the possible pathological substrates of such NAWM changes. We found that, in comparison with healthy subjects, MD of the NAWM was only modestly increased, whereas FA was markedly reduced, with a corresponding increase of FA histogram peak height, indicating a reduction of white matter fiber tract organization. This partial mismatch between MD and FA findings might be secondary to glial proliferation, which would lead to a "pseudonormalization" of MD values, but which would reduce FA values, since glial cells do not have the same anisotropic morphology as the tissue they replace. Albeit this hypothesis is not proven (and it would be hardly proven by pathologic studies, given the clinical evolution of this form of MS), the normality of NAWM MTR also points in the same direction, since the substitution of nerve tissue with glial cells is unlikely to modify the relative proportions of free and bound water in the brain, which in turn affect the MTR.

Table MTR, MD and FA histogram-derived metrics of the normal appearing white and gray matter from healthy volunteers and patients with early-onset MS

	Healthy volunteers	Early-onset MS patients	p
NAWM average MTR [%] (SD)	42.2 (1.8)	42.5 (1.8)	n. s.
NAWM peak height MTR (SD)	148.4 (26.7)	152.5 (33.4)	n. s.
GM average MTR [%] (SD)	38.2 (1.7)	38.2 (1.5)	n. s.
GM peak height MTR (SD)	103.4 (11.3)	103.6 (12.1)	n. s.
NAWM average MD [$\times 10^{-3}$ mm^2 s^{-1}] (SD)	0.83 (0.01)	0.86 (0.04)	0.02
NAWM MD peak height (SD)	182.0 (25.5)	177.1 (26.6)	n. s.
GM average MD [$\times 10^{-3}$ mm^2 s^{-1}] (SD)	0.98 (0.02)	1.00 (0.04)	n. s.
GM MD peak height (SD)	94.1 (16.6)	96.4 (21.4)	n. s.
NAWM average FA (SD)	0.29 (0.01)	0.26 (0.02)	< 0.0001
NAWM FA peak height (SD)	96.9 (5.7)	107.2 (13.2)	0.007

NAWM normal-appearing white matter; *GM* gray matter; *MTR* magnetization transfer ratio; *MD* mean diffusivity; *FA* fractional anisotropy; *SD* standard deviation; *MS* multiple sclerosis; *n. s.* not significant

Against this background, the next question to be addressed is why glial proliferation occurs in these patients. Again, this study can not give a definitive answer; however, the demonstration of a moderate correlation between diffusion changes and T2 lesion load allows us to propose that the 'occult' NAWM damage disclosed in these patients might be secondary to Wallerian degeneration of axons passing through focal, macroscopic lesions, which is then likely to cause glial proliferation. Since glial proliferation can be viewed as a reparative mechanism following tissue injury, an enhanced ability of patients with early-onset MS to repair might also contribute to their relatively favorable clinical outcome.

Finally and again contrary to what happens in MS with a classical onset in the adulthood [35], this study showed that brain atrophy does not seem to be a prominent feature of patients with early-onset MS. There is increasing evidence that brain atrophy is present from the earliest clinical stage of MS [35, 36] and it progresses over time with a yearly rate of 0.6–1 % in all adulthood disease phenotypes [35]. This, on the one hand, excludes the possibility that the NAWM diffusion changes are attributable to partial volume effects and, on the other, confirms that the MS-related damage is relatively mild in patients with early-onset MS. However, considering the cross-sectional design of this study and the lack of any correlation between brain volume and other quantitative MRI metrics, the role of brain atrophy in early-onset MS and its relationship with MRI metrics of NAWM and GM damage deserve further investigations. In addition, we can not rule out that gliosis might have prevented the detection of brain volume loss in these patients, and that brain atrophy might be found in a more advanced phase of the disease.

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