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ORIGINAL COMMUNICATION

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A study of the mechanisms of normal-appearing white matter damage in multiple sclerosis using diffusion tensor imaging

Evidence of Wallerian degeneration

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Abstract Diffusion tensor imaging (DTI) investigates brain tissue microstructure *in vivo*. In multiple sclerosis (MS) Wallerian degeneration of axons traversing focal lesions is a potential mechanism of damage in normal-appearing white matter. *In vivo* evidence for this hypothesis is limited. The present study investigated the relationship between DTI-derived indices in the normal-appearing corpus callosum (CC) and the lesion loads (LLs) in connected cerebral regions. DTI was performed in 39 MS patients and in 21 age-matched controls. Fractional anisotropy (FA) and mean diffusivity (MD) were estimated in the genu, body and splenium of CC. Patients showed lower FA and higher MD in the CC than controls and both correlated with the total LL ($r = -0.56$ and $r = 0.54$, $p < 0.0001$). The LL of individual cerebral lobes correlated with both FA and MD in the corresponding callosal regions, with the body showing the strongest correlations with frontal and parietal LL ($p < 0.0001$). The strong correlations between DTI indices in the CC and the extent of lesions in connected brain regions support the hypothesis that Wallerian degeneration of axons transected by remote, but connected focal lesions, is an important pathogenic mechanism of damage in MS.

Key words multiple sclerosis · diffusion tensor imaging · lesion load · axonal loss

Abbreviations

DTI = diffusion tensor imaging
CC = corpus callosum
LL = lesion load
FA = fractional anisotropy
MD = mean diffusivity

Introduction

Diffusion tensor imaging (DTI) is a sophisticated MRI technique that provides in vivo information about the pathological processes that modify brain microstructure [1]. Specific DTI indices can be used to describe the behaviour of water diffusion, including mean diffusivity (MD) [2], which measures the magnitude of diffusion, and fractional anisotropy (FA) [3], which quantifies the preferential directionality of water diffusion along white matter tracts.

We recently reported decreased FA and a trend toward increased MD in the normal-appearing white matter (NAWM) in patients with multiple sclerosis (MS) [4]. These diffusion changes in NAWM could reflect axonal loss with associated expanded extracellular space, which may result in a pattern of increased diffusivity and reduced directionality (anisotropy). This explanation is supported by post-mortem findings of a substantial loss of axons in the NAWM [5].

Of the different pathogenetic mechanisms that could cause a decrease in axonal density in the NAWM, Wal-

lerian degeneration of axons transected by MS lesions may play an important role [6, 7]. However, limited *in vivo* evidence of this potential mechanism has so far been reported [8, 9].

Thus, a key question is the extent to which NAWM pathology depends on the extent of focal lesion damage in MS. DTI has the potential to address this question by virtue of its sensitivity to fibre degeneration [10, 11]. By examining DTI-indices in the corpus callosum, in which there is a topographic and homologous arrangement of inter-hemispheric projections [12], it is possible to test whether lesion extent in individual lobes correlates with diffusion indices in connected callosal regions.

We therefore used whole-brain DTI to investigate *in vivo* the relationship between changes in the magnitude and anisotropy of water diffusion in the normal-appearing corpus callosum with the lesion loads in connected cerebral lobes in 39 patients with MS.

Methods

Patients

Thirty-nine patients with clinically definite MS [13] (11 benign, nine relapsing-remitting, 11 secondary-progressive, eight primary-progressive) [14] attending the National Hospital for Neurology and Neurosurgery, were studied. The mean age was 45 ± 11.2 years; the median Kurtzke expanded disability status scale (EDSS) [15] was 4.0 (range: 1.5–8.5); the median disease duration was 13 years (range: 3–33). There were 20 men and 19 women. Twenty-one healthy matched controls (mean age: 40.3 ± 9.7 years, 14 men and 7 women) were included in the study. Informed consent was obtained from all subjects before entry into the study.

MRI protocol

All scans were performed on a 1.5 T Signa Echospeed MRI system (General Electric, Milwaukee, USA). All patients had conventional spin echo Proton Density (PD) and T2 Weighted Images (WI) (TR 2000 ms, TE 30 ms, 120 ms; matrix size 256×256; FOV 240 mm; 28 × 5 mm axial slices). The diffusion protocol consisted of a whole-brain single-shot Diffusion Weighted Echo Planar Imaging (DW-EPI) [TE 78 ms; acquisition matrix size 96×96 reconstructed as 128×128; FOV 240 mm; 4 b values, increasing linearly with G^2 from 0 to 700 s mm^{-2}, applied along seven non-collinear directions]. The data were processed to determine the diffusion tensor on a pixel-by-pixel basis [1] and fractional anisotropy (FA) and mean diffusivity (MD) were calculated [3].

All images were displayed on a Sun workstation (Sun Microsystems, Mountain View, CA using the DispImage software package) [16]. Three square regions of interest (ROIs) of uniform size (9 pixels, 31.72 mm^2) were placed in the middle of the body, genu and splenium of CC. The ROIs were outlined on the non-diffusion weighted b0 (b = 0) images of the DW-EPI dataset, with guidance from the conventional PD and T2 WI to ensure that lesions and partial volume effects were avoided, and then automatically transferred to the FA and MD maps. The mean FA and MD for the whole CC were calculated from the three callosal regions.

Lesions were identified by a single observer (O. C.) blinded to the clinical details on the b0 images (with reference to the conventional PD-WI) using a semi-automated local thresholding technique [16]. The intra-observer coefficient of variation was less than 5%. The lesion loads (LLs) in each cerebral lobe and in the whole supratentorial brain were calculated on b0 images for each patient. Lesions of area < 20 mm^2 or in proximity to CSF were excluded to eliminate partial volume artefact.

Statistical analysis

The diffusion parameters were compared between patients and controls using the Mann-Whitney U test; the relationship between diffusion parameters and LL was assessed using Spearman's correlation coefficient (one-tailed).

Results

An example of ROIs located in the genu, body and splenium of CC on the FA and MD map is shown in Fig. 1. The differences in diffusion indices in each region of the CC are shown in Table 1. MS patients showed lower FA and higher MD in each callosal region considered separately compared with controls, although the statistical significance was reached only in the splenium. When the three callosal regions were combined, FA was significantly lower and MD was significantly higher in the whole CC than controls.

The median LL in the whole supratentorial brain was 11.58 cm^3 (range: 1.04–52.63); the largest LL was detected in the parietal lobes (median: 3.19 cm^3; range: 0.16–17.82), followed by the frontal LL (median: 2.76 cm^3; range: 0.14–17.26) and occipital LL (median: 1.14 cm^3; range: 0.0–19.92); the lowest LL was found in the temporal lobes (median: 0.69 cm^3; range: 0.0–10.77).

Correlations between the callosal regions and the LL in each cerebral lobe are reported in Table 2. The strongest correlations were found between FA and MD in the body of CC and LLs in all of the cerebral regions, in particular the parietal lobes. As might be expected from the callosal anatomy, frontal LL correlated with FA and MD in the genu and occipital LL correlated with FA and showed a trend with MD in the splenium. Frontal LL also correlated with MD in the splenium, whilst occipital LL correlated with MD in the genu. When the total brain LL and the whole CC were included in the analysis, there was a significant correlation between total LL and both FA ($r = -0.56$, $p < 0.0001$) and MD ($r = 0.54$, $p < 0.0001$) of the whole CC (Fig. 2).

Discussion

We investigated *in vivo* the relationship between the diffusion changes in three callosal regions and the lesion volume in connected cerebral areas using diffusion tensor imaging DTI [1], which quantifies the magnitude and anisotropy of water diffusion [2, 3].

The subtle involvement of the callosal white matter fibers independent of inflammatory lesions is well de-

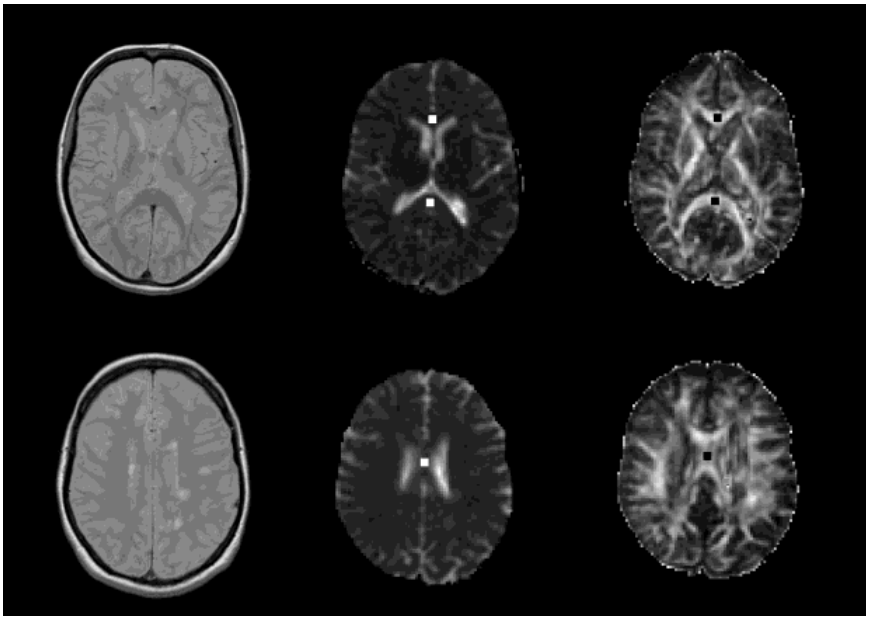

Fig. 1 Proton density weighted images, mean diffusivity (MD) and fractional anisotropy (FA) maps. ROIs are placed in the genu and splenium of corpus callosum, (first row) and in the body of corpus callosum (second row)

Table 1 Diffusion parameters for normal-appearing corpus callosum

Regions	FA controls Median (Range)	FA Patients Median (Range)	MD (x10^{-3} mm^2/sec) controls Median (Range)	MD(x10^{-3} mm^2/sec) patients Median (Range)
Body of CC	0.66 0.49–0.77	0.63 0.41–0.75	0.86 0.68–1.06	0.91 0.7–1.5
Genu of CC	0.77 0.62–0.88	0.76 0.53–0.9	0.82 0.64–1	0.84 0.63–1.4
Splenium of CC	0.73 0.63–0.89	0.66** 0.54–0.83	0.96 0.75–1.1	1.1*** 0.76–1.6
Whole CC (genu, body, splenium)	0.71 0.66–0.81	0.68* 0.54–0.78	0.88 0.80–0.98	0.92* 0.79–1.3

FA fractional anisotropy; *MD* mean diffusivity; *CC* corpus callosum
* significantly different from controls at Mann-Whitney U test ($p < 0.05$)
** significantly different from controls at Mann-Whitney U test ($p < 0.01$)
*** significantly different from controls at Mann-Whitney U test ($p < 0.001$)

Table 2 Relationship between lesion load in each cerebral lobe and diffusion indices in the callosal regions

	Frontal lesion load	Parietal lesion load	Occipital lesion load
FA genu	$r = -0.31$, $p = 0.027$	$r = -0.38$, $p = 0.009$	$r = -0.27$, $p = 0.05$
MD genu	$r = 0.39$, $p = 0.007$	$r = 0.42$, $p = 0.004$	$r = 0.32$, $p = 0.02$
FA body	$r = -0.54$, $p < 0.0001$	$r = -0.62$, $p < 0.0001$	$r = -0.5$, $p < 0.001$
MD body	$r = 0.52$, $p < 0.0001$	$r = 0.56$, $p < 0.0001$	$r = 0.42$, $p = 0.004$
FA splenium	$r = -0.19$, $p = 0.13$	$r = -0.19$, $p = 0.12$	$r = -0.28$, $p = 0.04$
MD splenium	$r = 0.28$, $p = 0.04$	$r = 0.18$, $p = 0.14$	$r = 0.25$, $p = 0.06$

scribed [17], but only recently has the extent of axonal loss in the CC been quantified by a pathological study [5]. Axonal loss, with associated expanded extracellular space [18], may result in increased diffusivity and reduced anisotropy in the normal-appearing CC of patients with MS.

The reason why the differences in diffusion parameters reached statistical significance only in the splenium is not clear, but it could be related to the differences in fiber composition of the CC [19]. Indeed thin fibers (smaller than 2 μm in diameter) are most dense in the splenium and smaller axons seem to be preferentially susceptible to injury in MS [20]. The reduction in the density of fibers passing through the CC could reflect

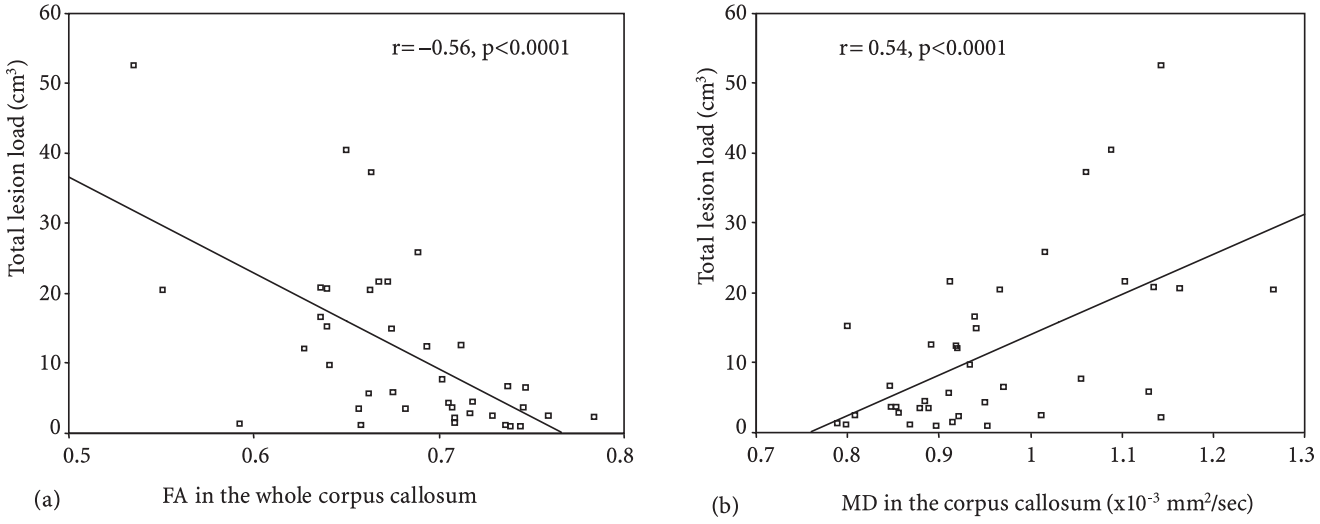

Fig. 2 Scatter plots of total lesion load versus fractional anisotropy (FA) (**a**) and mean diffusivity (MD) (**b**) in the corpus callosum. The lines represent the linear regression of the data

Wallerian degeneration of axons transected in remote MS lesions [6, 7]. This hypothesis has also been supported by a post-mortem study that has showed an inverse correlation between the regional lesion load (LL) and the total number of axons crossing the corresponding projection areas in the CC [21]. However, there are only limited data *in vivo* to suggest that the damage to axons traversing inflammatory lesions may be transmitted over long distances in the connected NAWM in patients with MS [8, 9].

The present study has shown significant correlations between the diffusion changes in the genu, body and splenium of CC with the LL in the cerebral lobes most connected to those regions (frontal, parietal and occipital lobes respectively), reflecting the anatomy of most callosal fibers, which interconnect homologous areas of both hemispheres [12]. The correlations between diffusion indices in the genu with the occipital LL, and those in the splenium with the frontal LL, could alternatively result from a minor contingent of callosal fibers terminating in areas of the controlateral hemisphere not homologous with their origin [12]. The strongest relationship between the cerebral LLs and diffusion indices in the body of the CC might be explained by a substantial number of fibers originating in the frontal and occipital lobes traversing the body of CC [22, 23].

These findings, together with the correlations between MD in the whole CC and the total LL, could however reflect other pathogenetic mechanisms than fibre degeneration. NAWM damage is known to involve more complex processes than simply axonal degeneration, including inflammation and astrogliosis [24]. The relationship between these pathological abnormalities occurring in MS, i. e. inflammation, demyelination, axonal damage and gliosis, is intricate. Acute axonal damage is secondary to axons transection in the setting of inflammatory demyelination [25], while progressive axonal degeneration may be related to lack of trophic support from oligodendroglia and myelin [26]. A recent spectroscopy investigation found a strong correlation between the total lesion load and the concentration of N-acetylaspartylglutamate (NAA) [27], which may indicate neuronal loss, in a spectroscopy volume, suggesting that an increased lesion burden anywhere in the brain is associated with the extent of axonal injury. Recent evidence has suggested that besides the axonal damage, the glial response may be clinically relevant (at least in the progressive phase) [28], and seems to precede secondary axonal degeneration [29]. A spectroscopy study reported a significant correlation between T2 lesion load and the myo-inositol concentration in the NAWM, suggesting that focal inflammatory activity is related to a widespread glial proliferation in the NAWM [30]. Another mechanism of damage to the NAWM could be related to the effect of diffusible factors associated with focal lesions [31]. Therefore, more than one pathogenetic mechanism may result in the NAWM damage and contribute to our correlations. Moreover, MD and FA findings may simply reflect the relationship between the overall extent of pathology in lesions and NAWM, rather than implying a specific mechanism of NAWM abnormalities. Indeed, a strong correlation between overall mean lesion MD and mean NAWM MD (without specific anatomically-based correlations) has been reported [32]. Taking in account these findings, the correlations between the callosal regions and the LLs may simply indicate that the pathological mechanisms are widespread and interrelated throughout the brain.

Since the LL was calculated on non-diffusion weighted $b = 0$ images, which are characterised by a

lower resolution than conventional T2 weighted images, the visualisation of small lesions or lesions located in the brain regions affected by geometric distortion could have been impaired [33]. This could potentially reduce the total LL in each cerebral lobe, or cause the preferential detection of lesions with the most pathological damage. Furthermore, a small number of lesions located within the callosal regions were not well visualized and were thus excluded from the analysis. However, the lesions in the CC were detected on the axial images, since the sagittal slices were not acquired in this study, and it could have contributed to incomplete definition of a small number of lesions.

In conclusion, DTI is sensitive to microstructural damage in the normal-appearing corpus callosum. Correlations of DTI indices (particularly anisotropy) with lesion extent in connected brain regions support the hypothesis that Wallerian degeneration of axons transected by remote, but connected focal lesions, is an important pathogenetic mechanism of normal-appearing brain tissue damage in MS.

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