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Regional DTI differences in multiple sclerosis patients

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ABSTRACT

Diffusion tensor imaging (DTI) measures have shown to be sensitive to white matter (WM) damage in multiple sclerosis (MS), not only inside focal lesions but also in user-defined regions in the so-called normal-appearing white matter (NAWM). New analysis techniques for DTI measures are now available that allow for hypothesis-free localization of damage. We performed DTI measurements of 30 MS patients selected for low focal lesion loads, and of 31 age-matched healthy controls and analyzed these using tract-based spatial statistics (TBSS). Patients were found to have a lower fractional anisotropy (FA) compared to controls in a number of brain regions, including the fornices, the left corona radiata, the inferior longitudinal fasciculus in both hemispheres, both optic radiations, and parts of the corpus callosum. In the regions of reduced FA, an increase in radial diffusivity and a less pronounced increase of axial diffusivity were found. Neurocognitive assessment showed that patients had normal visuospatial memory performance, just-normal attention, and impaired processing speed; the latter was associated with abnormal FA in the corpus callosum, an area which was relatively devoid of lesions visible on proton density-weighted images in our patients. TBSS can be useful in future studies with other MS patient samples to provide an unbiased localization of damage and generate location-specific hypotheses.

Introduction

In MS, part of the total WM damage is revealed by the T2 hyperintense lesions. These lesions reflect focal demyelination and axonal loss to variable extent (De Groot et al., 2001; Moore et al., 2000; van Waesberghe et al., 1999). However, pathology outside the focal WM lesions, in the so-called normal-appearing white matter (NAWM), remains largely undetected by conventional MRI. This is one of the main reasons why the association between MRI-visible abnormalities and MS clinical disability is only weak (Barkhof, 2002).

More advanced MRI techniques that have been used to quantify NAWM damage in MS include MR spectroscopy, whole-brain atrophy, T1 relaxation time measurements and diffusion tensor imaging (DTI) (Cader et al., 2007; Jasperse et al., 2007; Vrenken et al., 2006a). Overall disability and cognitive deficits were found to be related to these measures (De Stefano et al., 2001; Fisher et al., 2000).

Diffusion tensor imaging (DTI) is increasingly used in MS research (Rovaris et al., 2005). A diffusion tensor matrix can be generated for each voxel from acquired diffusion weighted images. From this matrix, secondary parameters such as fractional anisotropy (FA) can be calculated (Pierpaoli and Basser, 1996). FA provides information on the degree of diffusion directionality and ranges from 0 (isotropic diffusion) to 1 (anisotropic diffusion). FA is very sensitive since it is not only decreased in focal WM lesions but also in the NAWM (Bammer et al., 2000; Werring et al., 1999). However FA lacks specificity because various pathophysiological mechanisms may result in a decrease (Beaulieu, 2002).

From the diffusion tensor matrix, the three eigenvalues of diffusion can be derived. The first and largest eigenvalue is thought to reflect water diffusivity parallel to axonal fibers, and is referred to as axial diffusivity. The mean of the second and third eigenvalues, representing water diffusivity perpendicular to axonal fibers, is referred to as radial diffusivity. In animal studies, axial diffusivity was shown to be related to axonal damage, and radial diffusivity to demyelination (Song et al., 2003).

In the past, DTI measures of MS patients and controls have been compared using either summary measures of the whole-brain (Nusbaum et al., 2000), the NAWM (Filippi et al., 2000) or a region-of-interest (ROI) approach (Ciccarelli et al., 2001). The ability to spatially

Abbreviations: CC, corpus callosum; CIS, clinically isolated syndrome; CIS-20, Checklist of Individual Strength; DART, Dutch Adult Reading Test; DTI, diffusion tensor imaging; EDSS, expanded disability status scale; EHS, Edinburgh Handedness Scale; FA, fractional anisotropy; GM, gray matter; HADS, Hospital Anxiety and Depression Scale; ILF, inferior longitudinal fascicle; LDST, Letter Digit Substitution Test; LLT, Location Learning Test; LPM, lesion probability map; MS, multiple sclerosis; NAWM, normal-appearing white matter; ROI, region of interest; RR, relapsing-remitting; SP, secondary progressive; VBM, voxel-based morphometry; WM, white matter.

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localize group differences in MRI data in an observer-independent and hypothesis-free fashion has now become available with the emergence of new analysis tools, such as voxel-based morphometry (VBM) for T1-weighted structural MR data (Ashburner and Friston, 2000). To analyze diffusion tensor MR data in a voxelwise fashion, tract-based spatial statistics (TBSS) was recently developed (Smith et al., 2006). In contrast with VBM-style analysis, TBSS takes advantage of the spatial determinants of major white matter tracts and thereby minimizes registration errors (and bias), thus eliminating the need for arbitrary smoothing.

In this study, we used TBSS to localize areas of abnormal FA, compared with an age-matched healthy control group, in 30 MS patients with low total WM lesion loads. Furthermore, changes in axial and radial diffusivity were explored in areas of abnormal FA. In addition to MR imaging, all subjects underwent a neurocognitive assessment, and the relation between cognitive deficits and DTI-findings was explored.

Materials and methods

Subjects

Thirty patients (mean age±SD: 40.6±9.1 years; 19 females) and 31 age- and sex-matched healthy controls (mean age±SD: 40.6±9.9 years; 21 females) were included. The patients were selected for this study from a clinical MS database. Patients with large confluent lesions visible on previous MRI scans were excluded. The study protocol was approved by the institutional ethics review board and all subjects gave written informed consent prior to participation.

Magnetic resonance imaging

MR imaging was performed on a 1.5T whole-body scanner (Siemens Sonata, Erlangen, Germany), using an eight-channel phased-array head coil. Diffusion-weighted echoplanar images (TR 8500 ms, TE 86 ms; 59 contiguous axial slices with an isotropic 2 mm resolution; acquisition time 10 min) with 60 volumes with non-collinear diffusion gradients (b-value of 700 s mm^{-2}) and 10 volumes without directional weighting were acquired. Furthermore, turbo spin-echo proton density- and T2-weighted images were obtained (TR 3130 ms, TE 24/85 ms). The dual-echo images were interleaved and consisted of 46 axial slices with a slice thickness of 3 mm and an in-plane resolution of 1×1 mm^2.

Image analysis

All image manipulation tools used in this study are part of the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl). For the diffusion-weighted images, motion and eddy current distortion were corrected with FMRIB's Diffusion Toolbox (FDT), which was also used to fit the diffusion tensor for each voxel. From this tensor, FA and radial and axial diffusivity were derived voxelwise.

The TBSS pipeline firstly performed a search for the FA image of the 'most typical subject' of the dataset. After this, individual FA images were non-linearly registered to the FA image of this most typical subject. Subsequently an affine transformation was used to warp the FA images from the most typical subject space to standard space. Computing times of over 3000 h on a single processor to find the most typical subject were reduced to 70 h by performing this search on the grid infrastructure provided by the Virtual Laboratory for e-Sciences project (http://www.vl-e.nl). The FA images in standard space were averaged and the result, a mean FA image, was skeletonised. This mean FA skeleton image was thresholded at 0.2 to include only WM voxels. For each subject's registered FA image, the maximum FA value perpendicular to each voxel of the skeleton was projected onto this skeleton. The individual registration and projection vectors obtained during the above-described process were also applied to the radial and axial diffusivity data.

An experienced reader marked hyperintense WM lesions on the short-echo images of the T2-weighted sequence, in line with common practice in brain imaging studies. Subsequently, WM lesion volumes were measured using in-house-developed software.

In order to create a lesion probability map, T2-weighted images were linearly registered to standard space with an affine transformation. The resulting transformation matrices were used to co-register the individual lesion masks. Registered lesion masks from all patients were added to create a single map, which was averaged, resulting in a map with a value for each voxel ranging from zero to one, indicating the proportion of patients with a lesion in that particular voxel. To assess whether FA differences were colocalized with focal lesions, this map was thresholded at 0.1, thus showing voxels in which in at least three out of the total of thirty patients a lesion was present, and used as an overlay on the output image of TBSS showing significant areas of FA difference.

Clinical and neuropsychological assessment

All patients underwent a neurological examination on the day of scanning, from which the expanded disability status scale (EDSS) (Kurtzke, 1983) was determined. In addition, patients and controls underwent a neurocognitive assessment, designed to investigate the cognitive domains most frequently affected in MS. Attention and inhibition were assessed using the Stroop Interference test (Stroop, 1935). Spatial memory was evaluated with the Location Learning Test (LLT) (Bucks and Willison, 1997). The Letter Digit Substitution Test (LDST) (Jolles et al., 1995) was included to assess processing speed of visual information. Furthermore, anxiety and depression, fatigue, premorbid intelligence and handedness, which may possibly bias neurocognitive outcome, were evaluated to control for intrinsic differences between MS patients and controls where appropriate. Symptoms indicative of depression, anxiety and fatigue were assessed by questionnaires: the Hospital Anxiety and Depression Scale (HADS-D and HADS-A) (Zigmond and Snaith, 1983) and the Checklist of Individual Strength (CIS-20) questionnaire (Vercoulen et al., 1994). Pre-morbid intelligence was investigated using the Dutch version of the New Adult Reading Test (DART) (Nelson and O'Connell, 1978; Schmand et al., 1991). Lastly, handedness was evaluated using the Edinburgh Handedness Scale (EHS) (Oldfield, 1971).

Statistical analysis

Differences in FA between patients and controls were analyzed in a voxelwise fashion using FSL's *randomise* (which combines General Linear Model testing with permutation inference statistics). A cluster-forming threshold of $t=3$ and a corrected cluster size significance level of $p<0.05$ was used to correct for multiple comparisons. Changes in radial or axial diffusivity in areas of FA group differences were assessed by transformation of clusters of FA difference into ROIs, which were used to derive mean FA, mean radial and mean axial diffusivity per cluster for each subject. Values are reported as mean±SD, unless indicated otherwise.

Student's t-test was used for group comparisons when data was normally distributed, otherwise the Mann–Whitney U test was used. Correlations between DTI findings and clinical measures were only examined in the patient group, in this case Pearson's r was used. p values <0.05 were considered statistically significant.

Results

Subject descriptives

Subject group descriptives (Table 1) show that the control group was well matched for age and premorbid intelligence. In patients, the