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Active brain changes after initiating fingolimod therapy in multiple sclerosis patients using individual voxel-based analyses for diffusion tensor imaging

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ABSTRACT

Voxel-based analysis (VBA) of diffusion tensor images (DTI) and voxel-based morphometry (VBM) in patients with multiple sclerosis (MS) can sensitively detect occult tissue damage that underlies pathological changes in the brain. In the present study, both at the start of fingolimod and post-four months clinical remission, we assessed four patients with MS who were evaluated with VBA of DTI, VBM, and fluid-attenuated inversion recovery (FLAIR). DTI images for all four patients showed widespread areas of increased mean diffusivity (MD) and decreased fractional anisotropy (FA) that were beyond the high-intensity signal areas across images. After four months of continuous fingolimod therapy, DTI abnormalities progressed; in particular, MD was significantly increased, while brain volume and high-intensity signals were unchanged. These findings suggest that VBA of DTI (e.g., MD) may help assess MS demyelination as neuroinflammatory conditions, even though clinical manifestations of MS appear to be in complete remission during fingolimod.

Key Words: multiple sclerosis (MS), fingolimod, voxel based morphometry (VBM), diffusion tensor imaging (DTI), fluid-attenuated inversion recovery (FLAIR) high-intensity signals.

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated, inflammatory, and demyelinating degenerative central nervous system disease. Conventional magnetic resonance imaging (MRI) is useful for evaluating macroscopic lesions in MS; however, MRI is insensitive to potential microscopic lesions such as those in normal-appearing white matter. Voxel-based analyses (VBA) of diffusion

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tensor images (DTI) and voxel-based morphometry (VBM) are more sensitive to occult tissue damage compared with conventional MRI. These refined techniques should help to obtain additional information regarding underlying pathological changes.[1] Recent DTI studies have revealed decreased fractional anisotropy (FA) and increased mean diffusivity (MD) in patients with MS.[1,2] VBM studies have revealed both gray and white matter atrophy, as well as the mitigation of these changes during subsequent therapy.[3,4] However, these aforementioned studies were conducted using group-comparison methods between patients and controls and included longitudinal MRI scans over several years. Moreover, VBA using a statistical jackknife approach for comparing an individual subject with a control group[5] can provide assessments on an individual patient, particularly during follow-up tests.[6]

Fingolimod, a sphingosine 1-phosphate (S1P) receptor modulator, is one of the drugs for MS[8] which effectively suppress clinical progression and symptom relapse.[7] However, the immunosuppressive effect of fingolimod may take several months to manifest and may differ among individuals in both duration from onset and extent of its effect.[9] Thus, it is important to assess inflammatory and degenerating status in the MS brain during fingolimod. In the present study, we demonstrated VBA of DTI and VBM findings that provide information regarding concurrent pathological MS conditions, even during clinical remission post-fingolimod treatment.

SUBJECTS AND METHODS

Subjects were four patients with MS (two women, two men) referred to the Department of Neurology at Nagoya University. Demographic data and results from serological examinations are summarized in Table 1. Clinical diagnoses of MS were established using McDonald's criteria, and all were categorized with a relapsing-remitting disease course.[10] None of the patients had a medical history of stroke or traumatic brain injury, none fulfilled Wingerchuk's criteria,[11] and all were negative for serum antiaquaporin-4 (AQP4) antibodies,[12] which are representative markers for neuromyelitis optica. Before fingolimod treatment, none of the patients had been diagnosed as MS, and none of them received continuous steroids, injection of interferon beta-1a/b, or any other immunomodulating drug. All brain MRI scans were performed on the same 3.0 T instrument with the same sequences, both just before the initiation of fingolimod and four months later. We also assessed clinical and physical scores derived from the Expanded Disability Status Scale (EDSS),[13] as well as blood examinations, including white blood cell (WBC) and lymphocyte counts (Table 1). During the intervening four months of fingolimod treatment, no patient had MS attacks or exhibited worsened symptoms. We also studied 28 healthy controls: 14 men and 14 women with a mean age of 53.6 ± 7.2 years (range: 29–66 years). All patients and controls were Japanese, and they underwent the same MRI examination protocol. Informed written consent was obtained before participation. The ethics committee from the Nagoya University Graduate School of Medicine approved this study.

MRI protocol

3D T1-weighted images, 3D fluid-attenuated inversion recovery (FLAIR) images, and DTI data were acquired on a 3.0 T scanner (Trio Siemens, Germany). The patients' 3D-T1 and FLAIR images were carefully reviewed to exclude for any potential abnormalities as compared to control subjects. For T1-weighted images, 192 axial slices were obtained using the following parameters: TR: 1,570 ms, TE: 2.15 ms, inversion time: 800 ms, flip angle: 15°, acquisition matrix: 256 × 256, reconstruction matrix: 256 × 256, field of view (FOV): 256 × 256 mm, in-plane resolution: 1.0 × 1.0 mm^2, slice thickness: 1.0 mm, no gap. For 3D-FLAIR images, 192 axial slices were

Table 1 Demograpic and clinical characteristics of the patient sample

Characteristics	Case 1	Case 2	Case 3	Case 4
Age (years)	54	29	36	32
Gender	male	female	male	female
Primary symptom	myelopathy	opthalamic disorder	cerebral damage	myelopathy
Number of MS attacks before the study	3	2	2	2
Disease duration (years)	6	2	2	2
EDSS at 1st MRI	3.5	1.5	3.0	1.5
EDSS at 2nd MRI	3.5	1.5	3.0	1.5
WBC count at 1st MRI (/mm^3)	7000	4900	5000	7500
Lymphocyte count (/mm^3) / %	2000 / 28.5	1500 / 30.6	1680 / 33.5	1700 / 22.4
WBC count at 2nd MRI (/mm^3)	4500	3600	4200	4900
Lymphocyte count (/mm^3) / %	700 / 15.5	640 / 17.7	890 / 21.1	700 / 14.1

EDSS: expanded disability status scale score, MRI: magnetic resonance imaging, MS: multiple sclerosis, WBC: white blood cell.

obtained using the following parameters: TR: 5000 ms, TE: 437 ms, inversion time: 1,550 ms, flip angle: 15°, acquisition matrix: 256 × 256, reconstruction matrix: 256 × 256, FOV: 256 × 256 mm, in-plane resolution: 1.0 × 1.0 mm^2, slice thickness: 1.0 mm, no gap. Diffusion-weighted images were obtained by employing optimal methods using a Stejskal–Tanner sequence with a single shot spin-echo-type, echo-planar imaging, a flip angle of 90°, and a repetition time of 7,800 ms, with a 12-channel phased-array head coil. The echo time corresponding to the respective b-factor was 84 ms for 1,000 s/mm^2. Echo spacing was 0.69 ms, and the matrix size was 128 × 128 with a readout bandwidth of 1,776 Hz/pixel. Sixty-three axial slices, 2.0-mm thick with no interslice gaps, were used to image the entire brain with a FOV of 256 × 256 mm. A motion probing gradient (MPG) was applied in 12 orientations after the acquisition of b = 0 images. The 128 × 128 data matrix was not interpolated.

VBM data analyses

3D T1-weighted images were analyzed using Statistical Parametric Mapping (SPM8; Wellcome Department of Imaging Neurosciences, London, UK; http://www.fil.ion.ucl.ac.uk/spm) and VBM8 (Department of Psychiatry, University of Jena, Germany) with Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra (DARTEL) running on MATLAB (MathWorks, Natick, MA, USA).[14,15] To allow an unbiased comparison among regions of interest in different patients, images of gray and white matter were finally smoothed with an 8-mm isotropic Gaussian kernel.[14]

Voxel-based DTI

FA and MD maps in the analyze format were produced using a combination of software from dTV-II.SR (http://www.ut-radiology.umin.jp/people/masutani/dTV.htm) and Volume-One 1.72 (http://www.volume-one.org).[16] To allow for voxel-based statistical comparisons, the FA and MD maps were normalized using SPM8, and these normalized FA and MD maps were smoothed with an 8-mm isotropic Gaussian kernel, which was the same value in VBM method.[14]

Image analyses using SPM8

In order to assess the individual abnormal quantitative extents for each MS patient compared

with 28 healthy controls (14 men and 14 women with an average age of 53.6 ± 7.2 years, range 29–66 years) using VBM and DTI, a whole brain voxel-based jackknife approach was conducted.[5,6] This consists of repeating the SPM image statistical analysis, including each MS patient versus 28 controls, for the difference and repeating the analysis. The preprocessed data were analyzed using an analysis of covariance model; age and gender (total brain volume added for gray and white matter volumetric analyses) were considered nuisance variables. We measured the abnormal volumes by counting the significant abnormal clusters of gray and matter decreases, FA decrease, and MD increase volumes for all four MS patients. The statistical threshold for significance was $p < 0.001$ (uncorrected for multiple comparisons) with an additional cluster extent threshold of 50 voxels (gray and white matter volumes) or 20 voxels (FA and MD maps).

RESULTS

All four patients had no clinical progressions or relapses during the clinical remission period after fingolimod initiation. The FLAIR, VBM, and VBA of DTI data for each patient (Figures 1–4, Cases 1–4, respectively, and Table 2) showed widespread areas of increased MD, most prominently extended beyond the lesions detected by FA decreases. FLAIR high signal was restricted in the detected regions as compared with MD increases and FA decreases; however, extension of detected regions was highly variable among individuals. Patterns of reduced gray matter and white matter volumes were also different among the four patients, and the white matter reductions were mainly identified as adjacent to the ventricles and callosal body areas.

After four months of fingolimod treatment, the areas with DTI abnormalities were markedly progressed. Especially, the areas and degrees of increased MD were more widespread and stronger than those showing FA decreases across patients. However, changes differed between the four patients. For example, increased MD in Case 4 was most remarkable, and increased MD in Case 3 occurred within subcortical white matter regions, in addition to areas adjacent to the ventricles and callosal body as compared to other patients. No new abnormalities appeared on the FLAIR images, and total intracranial brain volumes, including both gray and white matter, were not remarkably reduced for the patients. Moreover, all MS patients maintained the same neurological manifestations during the four-month interval. No increased gray or white matter volumes, and no increased FA or decreased MD were identified for all MS patients compared with 28 healthy controls.

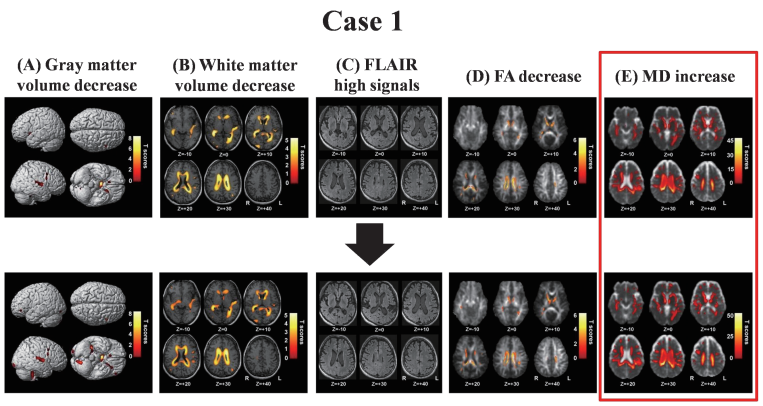

Figure 1

Figure 2

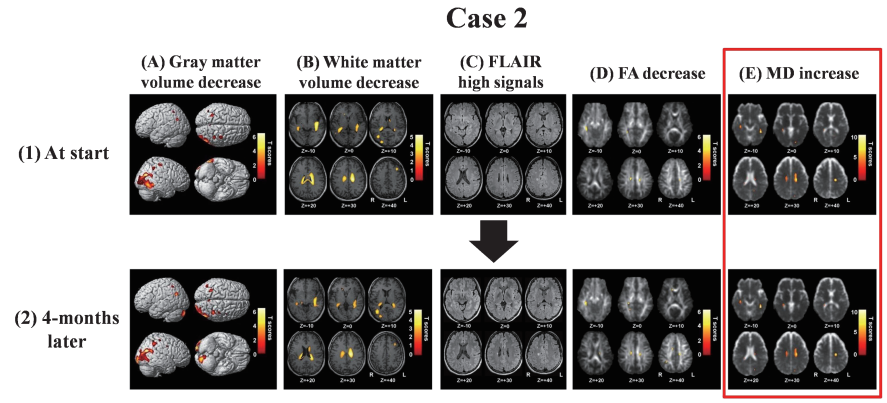

Figure 3

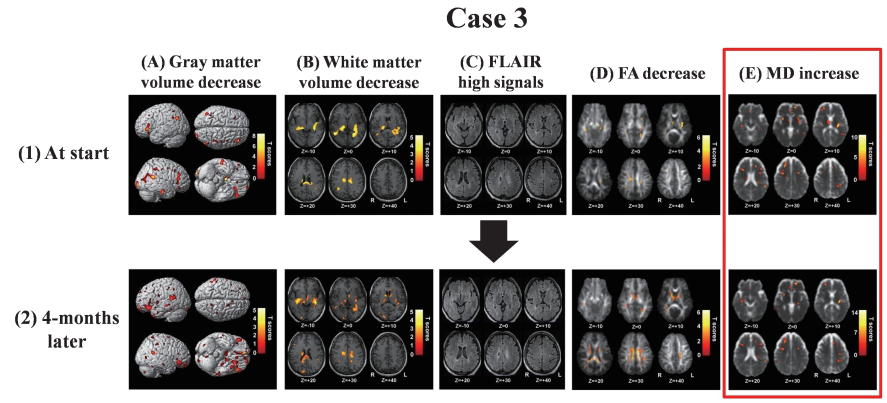

Figure 4

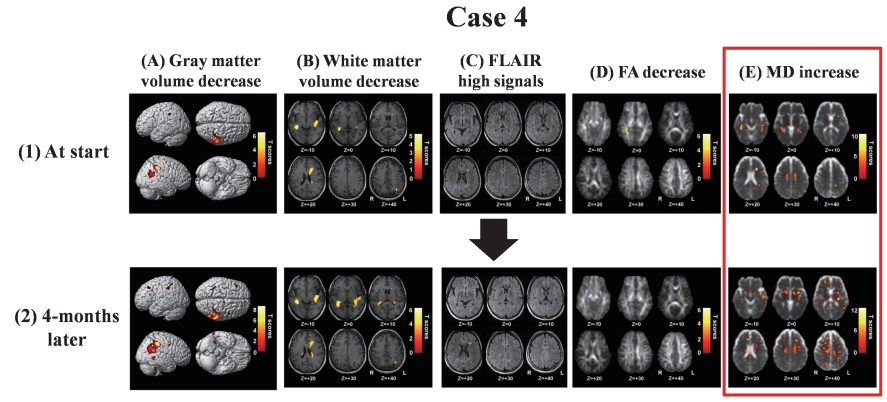

Fig. 1–4 Comparisons between individual patients with MS (Case 1–4) and 28 healthy controls (1) at the initiation of fingolimod treatment and (2) four months post-treatment. (A) FLAIR images (normalized to a T1-template created in SPM8). (B) Gray matter volume images showing areas of volume reduction with a T-score bar. (C) White matter volume maps showing areas of volume reduction rendered as FLAIR images with a T-score bar. (D) Fractional anisotropy maps showing areas of decreased anisotropy with a T-score bar. (E) Mean diffusivity maps showing areas of increased diffusivity with a T-score bar. All statistical analyses were set at $p < 0.001$, uncorrected at the voxel level.
FLAIR, fluid-attenuated inversion recovery; R, right; L, left.

Table 2 Abnormal volumes of cumulative lesions from patient MRI data

	Case 1	Case 2	Case 3	Case 4
Brain volumes (ml)				
GM volume; 1st MRI	712.040	783.354	743.063	749.167
GM volume; 2st MRI	709.575	775.797	740.359	746.582
WM volume; 1st MRI	378.601	484.134	473.839	458.716
WM volume; 2st MRI	373.504	479.461	468.708	456.855
Volume of DTI abnormal cumulative lesions (ml)				
FA decrease; 1st MRI	24.752	10.096	23.680	13.068
FA decrease; 2nd MRI	26.992	10.256	24.928	17.040
MD increase; 1st MRI	172.216	23.552	40.736	57.728
MD increase; 2nd MRI	197.760	30.024	50.032	153.024

Voxel-based imaging analyses with a statistical jackknife method compared with healthy controls (n = 28). DTI: diffusion tensor imaging, FA: fractional anisotropy, GM: gray matter, MD: mean diffusivity, MRI: magnetic resonance imaging, WM: white matter.

DISCUSSION

The present study used multimodal, voxel-based imaging analyses (that included a statistical jackknife method) to assess microstructural brain damage in patients with MS. The extent of DTI abnormalities, especially MD increases, was more extensive and widespread than FA decreases across all patients. This was similar to a previous observation in a group analysis assessing both controls and patients.[2] Our previous study also showed that MD was a more sensitive indicator of MS pathology than FA, high-intensity FLAIR signals, or volumetry.[1] Moreover, changes in MD correlated significantly with changes in patients' clinical characteristics.[1-2,17-18] The estimation of high-intensity lesions on FLAIR or T2-weighted images (FLAIR/T2) and brain volume measurements are widely used in clinical assessments of MS. However, these high-intensity lesions correspond to completely demyelinated lesions and brain atrophy, which are irreversible; these phenomena do not reflect the microstructural changes that occur in normal-appearing white matter.

According to previous studies, an increase in MD may be associated with a wide variety of pathological alterations, including demyelination and remyelination, axonal injury, astrocytic hyperplasia, edema, and perivascular infiltration as neuroinflammatory conditions,[19] while a decrease in FA mainly reflects demyelination, axonal injury, and the accumulation of inflammatory cells.[20] Based on these findings, increases in MD progression observed in the present study may be due to the deleterious effects of early pathological changes, including minimal edema and perivascular infiltration of the white matter despite clinical treatment.

The administration of fingolimod slows the progression of brain volume atrophy and restrains the new appearance of high-intensity lesions on FLAIR/T2 images. However, these image changes usually require a long time to detect therapeutic effects[21-23] and are not suitable to determine rapid treatment responses. The most striking results of the present study were that two of four patients, Case 3 and 4, exhibited active brain regions with DTI changes (especially increased MD) during fingolimod treatment, while the FLAIR images and VBM analyses did not reveal any changes. There were no changes in neurological symptomatic manifestations in these two patients with increased MD changes, suggesting that MD of DTI may be a highly sensitive marker to detect active regions in white matter for an individual patient. Another important observation

from this study was that the jack-knife method, particularly MD, may detect changes in brain lesions, distribution, and time-course for an individual MS patient. Taken together, these results indicate that jackknife analyses for MD may provide a beneficial method for detecting subtle but active neuroinflammatory lesions in patients with MS, even during a stable stage of clinical manifestation with initial fingolimod usage.

Pharmacologically, fingolimod has myriad influences that have not been fully elucidated. S1P has important signaling functions in the immune and central nervous systems. There are five G protein-coupled receptor subtypes (i.e., S1P1–5[24]), which are expressed differentially in certain cell types. The specific effects of fingolimod on subsets of lymphocytes could vary between individuals.[25] Similarly, fingolimod prevents lymphocytes from contributing to an autoimmune reaction through T helper 17 cells (Th17) in CD4-positive T cells. A recent immunological study reported that abnormalities in Th17 functioning are strongly connected to pathological changes in MS, including inflammation and tissue injury.[26] However, as discussed in the introduction, the immunosuppressive efficacy of fingolimod differs across individuals. Additionally, several months may be required for the onset of immunosuppressive effects of fingolimod.[9] Moreover, there have been anecdotal reports of MS relapses despite the initiation of fingolimod therapy. Many of these cases occurred within 1 year.[27,28] Similarly, the present study suggests that the observed differences in MRI changes after four months might be due to this very short treatment interval and treatment efficacy.

Limitations of the present study include the use of a relatively small patient sample and a short observation interval. Thus, longitudinal studies with larger patient samples and longer follow-up periods are keenly needed to further clarify the distribution of both gray and white matter pathology, as well as changes in disease course from relapsing-remitting to primary progressive, secondary progressive, or progressive relapse types. Additionally, our blood examinations included only white blood cell and lymphocyte counts. Future studies should examine serum compounds that contribute to Th17 formation.[29,30] In terms of our neuroimaging methods, voxel-based imaging analyses that are performed with a statistical jackknife method represent relative evaluations that involve comparisons with healthy controls. These methods do not directly measure the raw values of partial brain volumes and DTI values, including FA and MD. Nevertheless, the MD increases were more remarkable than any other MRI parameter in the present study. Thus, we speculate that evaluations of MD changes may be particularly useful for assessing brain conditions, especially discrepancies between clinical stabilization and the progression of brain inflammation from the viewpoints of degeneration with MS patients during these short intervals after fingolimod initializations, which are considered as disease modifying therapy to prevent the flare-ups of MS strongly.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest (COI).

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