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BRIEF REPORT

No Changes in Functional Connectivity After Dimethyl Fumarate Treatment in Multiple Sclerosis

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ABSTRACT

Introduction: Despite the increased availability of disease-modifying therapies (DMTs) for treating relapsing-remitting multiple sclerosis (RR-MS), only a few studies have evaluated DMT-associated brain functional changes.
Methods: We investigated whether significant resting-state functional connectivity (FC) changes occurred in RR-MS patients after 6 and 12 months of dimethyl fumarate (DMF) treatment using both a seed-based and data-driven approach.
Results: Thirty patients were followed up after 6 months of therapy, and 27 of them reached a 12-month follow-up. Three patients at baseline and only one after 12 months showed gadolinium-enhancing lesions. We did not find any significant FC changes after therapy at either time point. After 12 months of DMF, we observed relatively modest brain volume loss and a significant improvement in Paced Auditory Serial Addition Test 3 s and 25-Foot Walk Test scores.
Conclusion: The absence of FC changes could be due to the low degree of baseline inflammation in our patients, though we cannot exclude that more time may be required to observe such changes. No FC changes may reflect a beneficial effect of DMF therapy, as supported by conventional MRI findings and clinical improvement.

Keywords: Multiple sclerosis (MS); Resting-state functional MRI; Functional connectivity; Dimethyl fumarate (DMF); Disease-modifying therapy (DMT)

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Key Summary Points

We investigated whether significant functional connectivity changes occur in relapsing-remitting multiple sclerosis patients after 6 and 12 months of dimethyl fumarate treatment

No significant functional connectivity changes were found after 6 or 12 months of dimethyl fumarate treatment

After 12 months of therapy, relatively modest brain volume loss was found, along with a significant improvement in Paced Auditory Serial Addition Test 3 s and 25-Foot Walk Test scores

The lack of functional connectivity changes may be due to the low degree of baseline inflammation in our patient cohort, suggesting that more time may be required to observe such changes

The lack of functional connectivity changes, along with modest volume loss and clinical improvement, may reflect a positive phenomenon (i.e., stability)

INTRODUCTION

Despite the increased availability of disease-modifying therapies (DMTs) for treating relapsing-remitting multiple sclerosis (RR-MS), only a few studies have evaluated DMT-associated brain functional changes [1]. In two recent studies, we investigated resting-state functional connectivity (FC) changes after initial treatment with fingolimod in RR-MS [2, 3]. In the first study, we found a significant decrease in FC between the primary motor cortex and posterior cortical areas, which correlated with a significant improvement in information processing speed [2]. In the second study, we used a data-driven approach (independent component analysis, ICA) to investigate large-scale network DMT-related FC changes and observed that only patients who reached NEDA (no evidence of disease activity) status [4] showed significant within- and between-network FC changes after fingolimod treatment. Moreover, NEDA status was predicted by higher within-network FC at baseline [3].

Another first-line treatment for RR-MS is dimethyl fumarate (DMF), a widely used oral DMT licensed in 2013 on the basis of two positive phase III studies [5, 6], which showed that DMF significantly reduced the proportion of patients who had a relapse over 2 years, as well as the annualized relapse rate, the rate of disability progression, and the number of lesions on MRI. DMF suppresses inflammatory response and protects against nerve cell death [7].

We hypothesized that beneficial effects of DMF are associated with reduced FC, as we previously demonstrated in patients treated with fingolimod [2, 3]. Therefore, the present study aimed to investigate (1) whether FC changes occur after 6 and/or 12 months of DMF therapy and (2) whether FC changes, if present, are comparable to those observed after fingolimod therapy [2, 3].

METHODS

Participants

Thirty-nine RR-MS patients were recruited and underwent MRI scanning and clinical testing at Policlinico Umberto I, Sapienza University of Rome, Italy.

Inclusion criteria were: age range 18–-65 years; diagnosis of MS according to the revised McDonald 2010 criteria [8]; relapsing-remitting form; eligibility to be treated with DMF according to European Medicines Agency indications; right-handedness.

Exclusion criteria were: concomitant serious systemic disease, steroid administration and relapse within 2 months of study entry, prior history of other neurological or psychiatric disorders, and/or contraindications to MRI.

MRI and clinical/neuropsychological data were acquired 1–2 days prior to starting DMF (therapy start—Tst), after 6 months (T6m), and after 12 months (T12m).

At each time point, the following clinical/neuropsychological measures were collected: Expanded Disability Status Scale (EDSS) score and MS Functional Composite (MSFC) score with its sub-scores [9-Hole Peg Test (9-HPT), 25-Foot Walk Test (25-FWT), and Paced Auditory Serial Addition Test (PASAT) 3 and 2 s] and Symbol-Digit Modalities Test (SDMT) score. At the follow-up examination, disability progression with respect to baseline evaluation was defined as a 1.5-point increase for patients with a baseline EDSS score of 0, a 1-point increase for patients with EDSS scores from 1.0 to 5.0, and a 0.5-point increase for patients with EDSS scores equal to or higher than 5.5 [9].

Ethics Statement

This study was performed in accordance with the ethical code of the ethics committee of Azienda Policlinico Umberto I, Sapienza University of Rome (Ref. 2984/12.12.2013), and the Declaration of Helsinki. After approval from the ethics committee, written informed consent was obtained from all subjects.

MRI Acquisition

Images were acquired with a 3-T scanner (Siemens Magnetom Verio) and a 12-channel head coil designed for parallel imaging (GRAPPA). Participants were advised to avoid consuming psychoactive substances, such as tea or coffee, within 2 h prior to MRI scans. The following sequences were acquired:

- Blood oxygen level-dependent (BOLD) single-shot echo-planar imaging [repetition time (TR) = 3000 ms, echo time (TE) = 30 ms, flip angle = 89°, field of view (FOV) = 192 mm, 64 × 64 matrix, 50 contiguous axial slices 3 mm thick, 140 volumes, voxel size = 3 mm^3, acquisition time = 7 min 11 s], with all patients instructed to close their eyes and stay awake during the resting-state fMRI acquisitions;
- High-resolution 3D T1-weighted (T1-3D) MPRAGE sequence [TR = 1900 ms, TE = 2.93 ms, inversion time (TI) = 900 ms, flip angle = 9°, FOV = 260 mm, matrix = 256 × 256, 176 sagittal slices 1 mm thick, no gap];
- Dual turbo spin-echo, proton density (PD) and T2-weighted images (TR = 3320 ms, TE1 = 10 ms, TE2 = 103 ms, FOV = 220 mm, matrix = 384 × 384, 25 axial slices 4 mm thick, 30% gap);
- T1-weighted spin-echo sequence acquisition after administration of a gadolinium-based contrast agent (TR = 550 ms, TE = 9.8 ms, FOV = 240 mm, matrix = 320 × 320, 25 axial slices 4 mm thick, 30% gap).

MRI Analysis

Lesion and Brain Volume

Lesion volume was calculated on PD images using Jim 5.0 software (Xinapse System, Leicester, UK; http://www.xinapse.com).

Measures of global brain volume and gray matter (GM) volume at baseline (Tst) were obtained from lesion-filled T1-3D brain images using SIENAX, while measures of percentage brain volume change (PBVC) between Tst and T6m and between Tst and T12m were obtained using SIENA, part of FMRIB's Software Library (FSL) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki).

Functional Connectivity

Functional MRI data were preprocessed using FSL, v5.0.9. Single-subject preprocessing was performed using FEAT (FMRI Expert Analysis Tool) and included motion correction, non-brain substance removal, spatial smoothing, and high-pass filtering. At this stage, we also carried out both linear and non-linear registration to obtain functional-to-standard transformation matrices. Four-dimensional GM maps were obtained with the *feat_gm_prepare* tool and used as voxel-wise nuisance variables in subsequent statistical analyses. Preprocessing details are reported in [2, 3]. We performed a seed-to-whole-brain interregional regression analysis placing the seed on the right hand cortical representation (left primary motor cortex) [2] and an ICA analysis using MELODIC tool (Multivariate Exploratory Linear Optimized Decomposition into Independent Components)

[10] followed by dual-regression analysis [11]. Resting-state networks (RSNs) of interest were identified via spatial correlation coefficients using RSNs generated by Smith et al. [12] and Yeo et al. [13] as templates and then verified by expert visual inspection (CPi, NP, PP).

Subject-level difference maps (ΔFC maps) between T6m and Tst and between T12m and Tst were obtained for primary motor cortex FC maps and for each RSN and used for further group-level voxel-wise analyses.

Statistical Analyses

Statistical analyses of demographic, clinical, radiological, and neuropsychological parameters were performed using SPSS statistics software (version 22.0). Longitudinal changes in clinical, neuropsychological, and radiological parameters were estimated using Wilcoxon signed-rank test ($p < 0.05$ for null hypothesis rejection).

Primary motor cortex FC changes were investigated performing one-sample *t*-tests on ΔFC maps using FEAT. Age, sex, and GM maps were included as nuisance variables. *Z* statistic images were thresholded non-parametrically using clusters determined by $Z > 2.3$ and a (corrected) cluster significance threshold of $p = 0.05$ [14].

To investigate within-network FC changes, one-sample *t*-tests were performed on ΔFC maps, with age, sex, and GM maps as nuisance variables. Voxel-wise statistical analyses were performed with permutation-based non-parametric statistics using FSL Randomise permutation-based program with 5000 permutations [15]. Clusters were determined by using threshold-free cluster enhancement (TFCE) [16] and a family-wise error (FWE)-corrected cluster significance threshold of $p < 0.05$.

Between-network FC differences were investigated using FSLNets toolbox (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). After normalization of the extracted time courses of all RSNs identified in each subject, the time courses of artifactual components and components of no interest were regressed out of the individual data. Subject-wise correlation matrices of both full and partial correlations of all remaining RSN time courses were then created. Between-subject testing was then conducted across correlation values (*Z*-transformed) acquired for pairs of independent components. Between-network connectivity differences between timepoints were investigated using non-parametric paired testing, with a statistical significance threshold set at $p < 0.05$, FWE-corrected.

RESULTS

Clinical and Conventional MRI Data

Demographic and clinical characteristics of patients at Tst are reported in Table 1. Thirty patients were scanned at T6m and included in the analyses. Eleven were treatment-naïve, 17 switched from a previous first-line DMT, and 2 switched from a second-line DMT. The switch from a first-line DMT was due to inefficacy, while the switch from a second-line DMT (natalizumab) was due to progressive multifocal leukoencephalopathy risk. Three patients dropped out after T6m because of their unavailability to follow the protocol time schedule. Therefore, 27 patients were scanned at T12m.

Scores obtained on the clinical/neuropsychological assessment and radiological features at Tst, T6m, and T12m are reported in Table 2.

Table 1 Demographic and clinical characteristics of our cohort of MS patients ($N = 30$) at therapy start (Tst)

MS patients ($N = 30$)	
Demographic/clinical features	
Age	41.9 ± 10.2
Female/male, *n*	25/5
Disease duration, years	11.0 ± 8.2
Pts. with relapse in previous year, *n* (%)	4 (13)
Time since last relapse, days [range][§]	80 [68–360]
Treatment naïve, *n* (%)	11 (37)
First/second-line therapy, *n*	17/2

Values are reported as the mean ± standard deviation
[§]In patients with a relapse in previous year

Table 2 Scores obtained in the clinical/neuropsychological assessment and radiological features at therapy start (Tst), after 6 months (T6m), and after 12 months (T12m) of DMF treatment and statistical comparison results (Wilcoxon signed-rank test with threshold of $p < 0.05$)

MS patients	Tst ($N = 30$)	T6m ($N = 30$)	T12m ($N = 27$)	p^*	p^{**}
Clinical/neuropsychological scores					
EDSS score [median (range)]	1.5 [0.0–4.0]	1.5 [0.0–5.5]	1.5 [0.0–5.5]	ns	ns
Pts. with relapse under DMF, *n* (%)†	–	1 (3)	1 (4)	–	–
9-HPT dominant hand, s	20.0 ± 3.4	20.0 ± 3.0	19.5 ± 2.9	ns	ns
9-HPT non-dominant hand, s	21.4 ± 4.8	21.6 ± 4.0	21.0 ± 3.6	ns	ns
25-FWT, s	6.4 ± 1.4	6.5 ± 1.7	6.0 ± 1.4	ns	0.005
PASAT 3, s	39.8 ± 15.2	42.0 ± 13.4	43.5 ± 12.0	ns	0.022
PASAT 2, s	32.5 ± 13.1	33.4 ± 10.7	33.4 ± 12.8	ns	ns
SDMT	44.2 ± 12.3	44.1 ± 13.1	43.0 ± 13.6	ns	ns
Radiological features					
Brain volume (cm^3)	1,407 ± 259	–	–	–	–
Gray matter volume (cm^3)	743 ± 59	–	–	–	–
T2-lesion volume (cm^3)	8.997 ± 10.700	9.215 ± 10.948	9.770 ± 11.332	ns	ns
Percentage brain volume change (%)†	–	− 0.12 [− 1.83–1.88]	− 0.24 [− 2.72–1.77]	–	–
Pts with new/enlarging lesions under DMF, *n* (%)†	–	6 (20)	6 (22)	–	–
Pts with gadolinium-positive lesion, *n* (%)	3 (10)	1 (3)†	1 (4)†	–	–

Values are reported as the mean ± standard deviation or median [min–max]
s seconds, *ns* not statistically significant
9HPT 9-Hole Peg Test, *25FWT*, 25-Feet Walk Test, *PASAT* Paced Auditory Serial Addition Test 3 and 2 s, *SDMT* Symbol Digit Modalities Test
*Differences between Tst and T6m
**Differences between Tst and T12m
†Respect to baseline

After 6 months of DMF ($N = 30$), two patients showed disability worsening and six showed MRI activity (new/enlarged T2 or gadolinium-enhancing lesions). Of these six, one dropped out of the study. In the following 6 months, only one other patient showed a new T2 lesion and another one showed disability progression.

With respect to baseline, SIENA showed a median PBVC of − 0.12 [− 1.83–1.88] after 6 months and of − 0.24 [− 2.72–1.77] after 12 months of DMF.

No significant longitudinal changes were found with respect to clinical/neuropsychological or radiological scores after 6 months of treatment. After 12 months, there was significant longitudinal improvement in PASAT 3 s and 25-FWT scores (PASAT 3 s: $p = 0.022$; 25-FWT: $p = 0.005$).

Functional Connectivity

No significant longitudinal changes in primary motor cortex FC were found after either 6 or

12 months of therapy ($p < 0.05$, FWE-corrected).

ICA yielded 30 independent components. Of these, we identified ten components that showed the highest spatial correlation coefficients with RSN templates: the default mode ($r = 0.76$), dorsal attention ($r = 0.51$), left and right frontoparietal ($r = 0.64$ and $r = 0.61$, respectively), executive control ($r = 0.62$), visual ($r = 0.69$), cerebellar ($r = 0.49$), orbitofrontal ($r = 0.39$), auditory ($r = 0.62$), and sensorimotor ($r = 0.59$) networks.

No significant longitudinal FC changes in within- or between-network FC were found after either 6 or 12 months of therapy ($p < 0.05$, FWE-corrected).

DISCUSSION

In the present study, we investigated possible FC changes after an initial 6-month treatment with DMF and after a longer follow-up period of 12 months. When applying the same methodological approach as in our previous studies [2, 3], we did not find any significant FC changes after DMF treatment. After 12 months of DMF, we observed relatively modest brain volume loss and a significant improvement in PASAT 3 s and 25-FWT scores. One possible explanation for the absence of any significant FC changes after therapy is the low degree of baseline inflammation at baseline. In fact, gadolinium-enhancing lesions were present in only three patients at therapy start, whereas in our previous studies on fingolimod effects [2, 3], 15 out of 30 patients showed gadolinium-enhancing lesions. Intrinsic neural activity is generally regulated by excitatory/inhibitory balance, which is closely related to the levels of glutamate and gamma-aminobutyric acid [17]. In the acute MS stage, a large quantity of glutamate is produced by activated leukocytes, macrophages, and microglia [18], as also demonstrated by MR spectroscopy [19]. Due to glutamate excitatory action, it is reasonable to assume that acute inflammation in MS may be linked to increased neural activity [20, 21], which may be reduced by anti-inflammatory drugs. The hypothesis that the effect on FC might be related to inflammation *prior* to therapy initiation is supported by evidence in the literature: the effect of DMTs on brain plasticity is more pronounced in patients who have a greater degree of central nervous system inflammation [22].

It is also possible that DMF needs more time to modulate FC, especially in the presence of a relatively low number of patients with active inflammation. A recent 2-year study on fingolimod effects on FC [23] showed no DMT effect until the second year. The authors ascribed this delayed effect to the inclusion of patients without active inflammation at therapy start.

Lastly, we cannot exclude the possibility that the lack of FC changes after therapy may reflect a positive phenomenon, i.e., stability, as conventional MRI and clinical data suggest. As a matter of fact, after 12 months of therapy we observed modest brain volume loss (− 0.24%), which was inferior to the reference annual rate of brain volume loss in MS patients (from − 0.4 to − 1.0%) [24, 25]. This result is consistent with a previous study on DMF-treated MS patients, which suggested a treatment effect on GM atrophy after 1 year of therapy [26].

We also observed an improvement in PASAT 3 s and 25-FWT scores. PASAT improvement may be due to a practice or learning effect; this seems to be supported by the absence of improvement in SDMT, which is less prone to such effects [27]. Regarding walking speed, the observed improvement in 25-FWT scores (6.25%) may not be clinically meaningful (an improvement $\geq$ 20% is generally considered as such); however, it is surely indicative of clinical stability [28].

Future studies with larger sample sizes and longer follow-up observations are needed to confirm the present findings and draw firm conclusions about the effects of DMF therapy on neural functioning in MS patients.

Study Limitations

One of the main limitations of the present study was the relatively small sample size ($N = 30$). However, we were previously able to detect

significant FC changes after 6 months of fingolimod treatment in 30 RR-MS patients who were scanned using the same scanner and sequences as those described in the present study and who had similar clinical characteristics to those of the present patient cohort [2, 3]. Furthermore, it has been demonstrated that even a smaller number of patients ($N = 24$) can show significant functional changes after oral DMT (interferon beta) [22].

Another main limitation was the lack of a control group of healthy subjects or a group of untreated patients. Although comparison with healthy subjects would have allowed us to characterize any FC changes as patient specific, previous studies have shown that MS patients and healthy subjects have different baseline connectivity in most RSNs [29]. The longitudinal study design was also meant to compensate for the lack of an untreated MS patient group, which would be unethical.

Lastly, the anti-inflammatory effects of DMF were evaluated only by means of conventional MRI. The use of other techniques, such as positron emission tomography, would have assessed the inflammation of brain tissue outside the lesion areas [30]. Furthermore, it is known that even when the blood-brain barrier is intact, infiltrates of activated microglia and macrophages, an expression of chronic inflammation, have been demonstrated in patients with MS and have been related to clinical disability [31]. Thus, we cannot exclude the presence of neuroinflammation in our patient sample, even in the absence of gadolinium-enhancing white matter lesions.

CONCLUSION

We did not observe FC changes after 6 months of DMF therapy, unlike our previous findings in fingolimod-treated patients [2, 3]. Furthermore, we did not observe FC changes after a longer follow-up period of 12 months. This could be explained by the low degree of baseline inflammation in our patients, suggesting that more time may be required to observe FC changes. An alternative explanation, also supported by conventional MRI findings and clinical improvement, is that the absence of FC changes reflects the absence of (further) impairment to the functional network, which can be regarded as preservation of brain function.

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conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Compliance with Ethics Guidelines. This study was performed in accordance with the ethical code of the ethics committee of Azienda Policlinico Umberto I, Sapienza University of Rome (Rif. 2984/12.12.2013), and the Declaration of Helsinki. After approval from the ethics committee, written informed consent was obtained from all subjects.

Data Availability. The datasets generated during and/or analyzed during the current study are not publicly available because of patient confidentiality and participant privacy restrictions. Requests to access the datasets should be directed to petsas@gmail.com.

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