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Effect of fingolimod on diffuse brain tissue damage in relapsing-remitting multiple sclerosis patients

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ABSTRACT

Background: Multiple sclerosis (MS) affects all areas of the brain resulting in both focal and diffuse damage. In Phase 3 clinical trials, fingolimod showed significant reductions in both focal lesions and rate of brain volume loss (BVL) in patients with relapsing-remitting MS.
Objective: To investigate if the effects of fingolimod 0.5 mg on BVL are mediated exclusively through its effects on focal damage or if fingolimod also acts independently in reducing diffuse damage.
Methods: This was a pooled post-hoc analysis of patients from two Phase 3 studies (FREEDOMS [N=1272] and FREEDOMS II [N=1083]), with no evidence of focal disease activity as defined by absence of gadolinium-enhancing lesions at baseline and new active lesions and clinical relapses at follow-up. The percent brain volume change (PBVC), as a measure of diffuse tissue damage, was assessed at Month (M) 12 and M24 by using the Structural Image Evaluation using Normalization of Atrophy (SIENA) method. A regression analysis was performed in the pooled intent-to-treat (ITT) population to quantify the treatment effect of fingolimod on BVL vs. placebo (PBO) in the overall population (unadjusted model), and whether this effect is sustained after adjusting for new active lesions and on-study relapses (adjusted model).
Results: Of 1088 patients, 638 (PBO, n=127; fingolimod, n=511) at M12 and 450 patients (PBO, n=68; fingolimod, n=382) at M24 showed no focal activity. Fingolimod significantly reduced PBVC by 65.5% over 12M (fingolimod vs. PBO: −0.16 vs. −0.45; p=0.001) and by 48.2% over 24 M (−0.42 vs. −0.81; p=0.004). An absolute difference in PBVC of −0.27% (p < 0.001) in favor of fingolimod vs. PBO over 24M was still evident in the pooled ITT population, after adjusting for active lesions and on-study relapses. The regression model suggests that 54% (−0.27%/−0.51%) of effects of fingolimod on PBVC are independent of its effects on visible focal damage.
Conclusions: The effect of fingolimod on diffuse damage is partly independent of its treatment effect on focal damage, suggesting that both inflammatory and neurodegenerative components of MS are affected.

1. Introduction

Multiple sclerosis (MS) is traditionally seen as an inflammatory disease of the central nervous system (CNS) that is characterized by the presence of circumscribed demyelinated plaques in the cerebral white matter (WM) (Kutzelnigg et al., 2005; Love, 2006). Recent post-mortem work has re-emphasized, however, that focal WM lesions are only part of the spectrum of MS pathology (Kutzelnigg and Lassmann, 2014). Alterations are also present in the so-called normal-appearing WM and in the grey matter (Vrenken and Geurts, 2007), confirming earlier pathologic observations that the disease process affects not only myelin, but also axons and neurons (Kutzelnigg and Lassmann, 2014; Vrenken and Geurts, 2007; Lassmann et al., 2007).

Magnetic resonance imaging (MRI) is widely used in the management of MS patients owing to its high sensitivity in detecting focal WM abnormalities. More recently, numerous studies have used MRI-based methods for a computed estimation of the brain volume loss (BVL) that accumulates throughout the course of

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the MS (Filippi et al., 2012). Indeed, these volumetric changes have shown great clinical relevance, due to their close correlation with patients' physical disability and cognitive impairment (Roosendaal et al., 2011; Sumowski et al., 2014). MRI has been used to monitor the effects of disease-modifying therapies (DMTs) on brain volume (De Stefano et al., 2014).

Fingolimod, a sphingosine-1-phosphate (S1P) receptor modulator, has been shown in three phase 3 studies to significantly reduce BVL, in addition to the significant effects on MRI measures and on clinical endpoints in RRMS patients (Calabresi et al., 2014; Cohen et al., 2010; Kappos et al., 2010). Moreover, a recent post-hoc analysis has suggested a complementary role of MRI lesions and BVL as potential surrogates for disability in the short term of clinical trials with fingolimod (Radue et al., 2015; Sormani et al., 2015) supporting the inclusion of BVL as one of the key outcome measures in the evaluation of therapeutic effects in RRMS (Calabresi et al., 2014; Kappos et al., 2010).

In this context, it would be important to further evaluate the extent to which the effects of fingolimod on BVL are mediated through its well-described impact on focal damage (relapses and MRI lesions) alone, or by an additional effect on the diffuse damage in the non-lesional tissue. To explore this, we performed two different post-hoc analyses on the pooled data from the two phase 3, double-blind, randomized, FREEDOMS and FREEDOMS II trials: (1) we assessed the percent brain volume change (PBVC), as a marker of BVL, in a sub-group of patients with no evidence of focal disease activity, defined as absence of both clinical relapses and active lesions; (2) we quantified the treatment effect of fingolimod versus placebo that is mediated exclusively by PBVC measures in a statistical model using the pooled population of the two trials.

2. Methods

2.1. Patients and study design

Data from both FREEDOMS (N=1272) and FREEDOMS II (N=1083) were pooled in the post-hoc analysis. FREEDOMS and FREEDOMS II (registered with ClinicalTrials.gov identifier NCT00289978 and NCT00355134), were placebo-controlled, double-blind, randomized, phase III studies in relapsing-remitting MS (RRMS). The study designs were similar and the inclusion/exclusion criteria of both trials have been previously described (Calabresi et al., 2014; Kappos et al., 2010). Briefly, RRMS patients aged 18–55 years, with a score of 0–5.5 on the Expanded Disability Status Scale (EDSS) and having one or more relapses in the previous year and/or two or more relapses in the previous two years were included. Eligible patients were randomized (1:1:1) to receive fingolimod 0.5 mg/day, 1.25 mg/day or placebo for two years. Data of the fingolimod 0.5 mg/day, 1.25 mg/day groups were pooled. The trials were both conducted in accordance with International Conference on Harmonization Guidelines for Good Clinical Practice and the ethical principles of the Declaration of Helsinki. The ethics committees and institutional review boards of all participating centers approved the study protocols. All participants provided written informed consent before any study-related procedures were performed.

2.2. Statistical analyses

For the purpose of this study we performed two types of analyses on pooled data from the two trials:

Analysis 1: We assessed the PBVC, in a sub-group of patients with no evidence of focal disease activity, defined as absence of clinical relapses, gadolinium enhancing (Gd+) T1-weighted lesions at study entry and new active lesions (Gd+ T1 lesions and/or new/enlarging T2 lesions) during the follow-up period. In both trials, PBVC was measured by SIENA (Structural Image Evaluation using Normalization of Atrophy) (Smith et al., 2002) at Month 12 and Month 24. This subgroup analysis was conducted to determine if there is a difference in BVL between fingolimod-treated and placebo patients, who did not have evidence of focal disease activity. PBVC at Month 12 and at Month 24 in the subgroup with no activity was compared between treatment arms using an ANOVA model.

Analysis 2: A regression analysis was performed on the pooled population of the two trials with complete data on MRI lesions, relapses and PBVC over a 24-month follow-up period, in order to confirm and quantify the treatment effect of fingolimod on BVL versus placebo, which are independent from its effect on focal disease activity.

Firstly, an unadjusted regression model was used with treatment as the only factor to define the overall treatment effect of fingolimod on PBVC over 2 years (vs. placebo). Secondly, an adjusted regression model with treatment, relapses (Yes/No) and active lesions (Yes/No) during the treatment period as factors was used to determine treatment effect on PBVC after adjusting for on-study relapse and lesion activity. In this way, the relative difference in the treatment effect between the unadjusted and the adjusted model represents the effect of fingolimod on PBVC, which is independent of its effect on MRI lesions and relapses.

3. Results

Analysis 1: Of the pooled patients from FREEDOMS and FREEDOMS II, a subgroup of 638 patients (placebo, n=127; fingolimod, n=511) over a total of 1996 patients with a PBVC assessment at

Table 1
Baseline characteristics.

Characteristic	Patients with no focal activity at Month 12 (N=638)	Patients with no focal activity at Month 24 (N=450)
Age, years	41.2 ± 8.5	41.5 ± 8.5
Women, (%)	76	74
Time since first symptoms of MS	10.3 ± 8.3	10.3 ± 8.1
No history of disease-modifying treatment (%)	40	38
Relapses within previous 2 years, n	2.02 ± 1.29	1.98 ± 1.21
EDSS score (median, range)	2.0 (0–5.5)	2.0 (0–5.5)
T2 lesion volume, mm^3	3526 ± 5356	3344 ± 4954
T1 hypointense lesion volume, mm^3	1181 ± 2479	1094 ± 2113
Normalized brain volume, cm^3	1519 ± 79	1519 ± 79

Data are mean ± SD unless otherwise stated.
EDSS, Expanded Disability Status Scale; *MS, multiple sclerosis*; *SD, standard deviation.*
Patients pooled from FREEDOMS and FREEDOMS II.

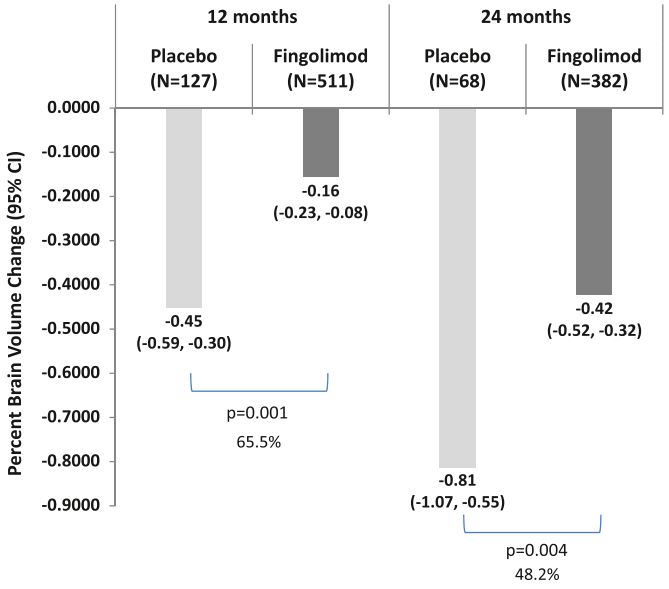

Fig. 1. Subgroup analysis: Fingolimod treatment effect on percent brain volume change in patients with no baseline Gd+ lesions, no relapses and no new active lesions, at 12 and 24 months. *Includes patients treated with both doses* (0.5 mg and 1.25 mg), *CI, confidence interval.*

Month 12, and 450 patients (placebo, n=68; fingolimod, n=382) over a total of 1799 patients with a PBVC assessment at Month 24, showed no focal disease activity. The baseline characteristics are reported in Table 1. In patients with no focal activity, fingolimod-treated patients had a significant reduction in PBVC over 12 months (−0.16% BVL in fingolimod group vs. −0.45% in placebo group, difference=−0.29%, 95%CI=[−0.13%; −0.46%], p=0.001) and on PBVC over 24 months (−0.42% in fingolimod group vs. −0.81% in placebo group, difference=−0.39%, 95%CI=[−0.12; −0.66%], p=0.004) vs. placebo patients (Fig. 1).

Analysis 2: In the pooled population with complete data on MRI lesions, relapses and PBVC over the follow up period (placebo, n=577; fingolimod, n=1198), the unadjusted regression model estimated the absolute difference in PBVC between fingolimod versus placebo at Month 24 to be −0.51% (–0.79% vs. −1.30%; p<0.001; Fig. 2). A significant effect on PBVC in favor of fingolimod was still evident when the model was adjusted for active lesions and on-study relapses, resulting in an absolute difference of −0.27% (p<0.001). The regression model therefore suggests that 54% (−0.27%/−0.51%) of the effects of fingolimod on PBVC, used here as a measure of BVL, are not directly the result of reducing lesions and relapses.

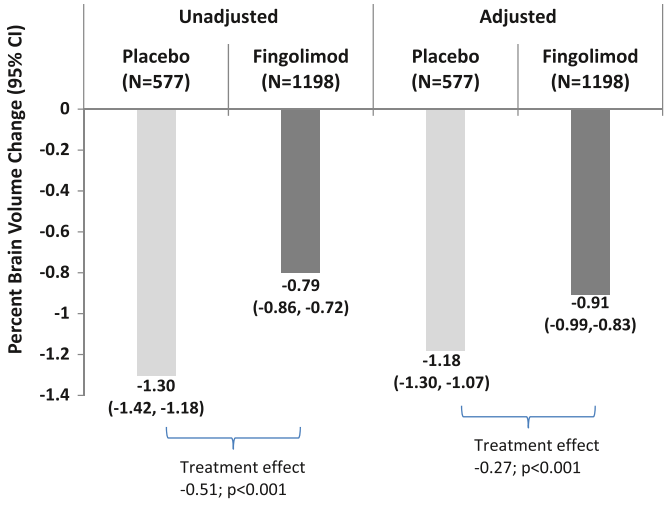

Fig. 2. Regression analysis: Fingolimod treatment effect on diffuse damage is still apparent when adjusting for focal damage. *Includes patients treated with both doses* (0.5 mg and 1.25 mg), *Adjusted for new active lesions and on-study relapses, CI, confidence interval.*

4. Discussion

BVL is a continuous process, occurring throughout the disease course of MS, at rates higher than in non-MS subjects (Bermel and Bakshi, 2006). BVL correlates well with both current and future disability and cognitive decline and, as such, has the potential to be used as an outcome measure for evaluating DMTs in the treatment of MS (Radue et al., 2015; Bermel and Bakshi, 2006). In Phase 3 studies of fingolimod, BVL correlated with MRI lesions, relapses and disability progression. However, the MRI lesions and relapses that represent focal disease activity and damage, accounted only for up to 50% of variability in brain volume changes, with the rest remaining unexplained, potentially reflecting undetected, diffuse damage (Calabresi et al., 2014; Radue et al., 2015).

In this post-hoc analysis, we assessed whether the treatment effect of fingolimod on BVL could be related, at least in part, to its effect on diffuse tissue damage. The main working hypothesis was, if MS patients without MRI and clinical signs of focal damage in the placebo and the treated arms still show a difference in BVL, it is likely that this effect is due to fingolimod's activity on pathological processes affecting non-lesional tissue. Furthermore, we assessed the 'residual' treatment effect of fingolimod on BVL, not explained by measurable focal disease activity, by comparing unadjusted and adjusted models for relapses and new/enlarging T2 lesions. The results of both analyses clearly showed that there is a significant portion of the treatment effect of fingolimod that is not explained by its effect on lesions/relapses and may be associated exclusively to diffuse BVL.

Several lines of evidence suggest that MS is not simply a focal demyelinating disease and that macroscopic lesions are just the tip of the iceberg of MS pathology. Indeed, the normal-appearing brain is profoundly abnormal, with a diffuse pathological process that appears to be distributed throughout the whole central nervous system (Kutzelnigg and Lassmann, 2014; Seewann et al., 2009). In such a context, the diffuse damage seems to occur, at least in part, independently from pathological changes within WM plaques (Kutzelnigg et al., 2005; Airas et al., 2015). This strongly suggests that the brain of MS patients is affected by pathological changes in a more global sense (Kutzelnigg and Lassmann, 2014).

Numerous neuroimaging studies have used MRI-derived methods to assess BVL in MS as a measure of diffuse tissue damage (Filippi et al., 2012; Radue et al., 2013). They have consistently shown that BVL can be observed from the earliest stages of MS and accumulates steadily over the course of the disease. Although in most studies significant BVL has been interpreted as largely due to the neurodegenerative processes occurring in MS (Barkhof et al., 2009), it must be stressed that, in a complex disease such as MS, this may reflect different pathological substrates of both neuroinflammatory and neurodegenerative origins. They certainly include (i) the shrinkage of WM lesions due to the loss of myelin, oligodendrocytes and axons and the contraction of astrocyte volume occurring during lesion maturation (ii) the neuronal and glial loss in cortical grey matter (GM) lesions and (iii) the Wallerian degeneration resulting from axonal transection in WM and GM lesions. The progressive volume loss occurring throughout the whole brain, however, is likely also the consequence of the diffuse inflammation, microglia activation and axonal injury occurring in the normal-appearing brain independently from focal demyelination. Interestingly, in preclinical studies fingolimod has

demonstrated the potential to act on at least some of these mechanisms, augmenting remyelination after toxin-induced demyelination, enhancing differentiation of oligodendrocyte progenitor cells and helping survival of mature oligodendrocytes, reducing astrogliosis and diminishing microglial activation (Airas et al., 2015; Chun and Brinkmann, 2011; Cui et al., 2014).

In the present analysis, we made an attempt to disentangle the complex RRMS pathology. Results clearly showed that a significant portion of the treatment effect on BVL is present independently of its effect on clinical and MRI measures of active, focal damage. Whether this is due to a direct, neuroprotective effect of fingolimod via S1P receptor modulation on neural cells (Cui et al., 2014; Colombo et al., 2014; Groves et al., 2013), and/or due to its immune-mediated effects leading to the interruption of the destructive link between inflammation and neurodegeneration and pro-inflammatory S1P signaling cascade in the CNS partly involving astrocytes (Kutzelnigg and Lassmann, 2014; Brinkmann et al., 2010; Frischer et al., 2009; Centonze et al., 2010), cannot be established here.

5. Conclusion

By showing a significant treatment effect of fingolimod on BVL, which is independent of its effect on MRI lesions and relapses, the present study provides new evidence of the important paradigm shift that has taken place in our understanding of the disease process in MS: the disease is not only due to focal inflammatory WM lesions, but involves more subtle and diffuse damage throughout the whole brain. This leads to the immediate need of targeting MS treatment not only to focal inflammatory lesions but also to the neurodegeneration that occurs. In this context, MRI-based measurements of brain volume are paramount to assess and monitor the effects of DMTs that could meet this target.

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