

Short-term brain volume change in relapsing–remitting multiple sclerosis

Effect of glatiramer acetate and implications

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Summary

The assessment of brain volume change with serial MRI provides an objective measure of an important component of the pathology of multiple sclerosis. Glatiramer acetate (GA) has a beneficial effect on clinical and MRI measures of disease activity and burden in patients with relapsing–remitting (RR) multiple sclerosis. This study investigated the impact of GA treatment on the development of brain atrophy in RR multiple sclerosis patients. The study consisted of a 9-month, double-blind, placebo-controlled phase followed by a 9-month open-label phase. Patients were randomized to receive either 20 mg GA or placebo by daily subcutaneous injections and underwent brain MRI scans every month during the first phase, and every 3 months during the second phase of the study. Using a semi-automated segmentation technique based on local thresholding, brain volume was measured from seven contiguous periventricular slices from the scans obtained at baseline, the end of the double-blind phase and the end of the study. From the original trial cohort, image

sets from 113 out of 119 patients randomized to GA, and 114 out of 120 randomized to placebo treatment were evaluated. Brain volume was significantly correlated with patients' disability at each time-point. No significant differences between placebo- and GA-treated patients were found for baseline brain volume and rate of brain volume change over the study, even though a possible late trend for treatment with GA to retard the loss of brain volume was observed. There was a significant, but modest, correlation between MRI activity during the double-blind phase, and brain volume change over the entire study among patients originally treated with placebo. The modest correlation between enhancement frequency and brain atrophy loss indicates that the suppression of inflammatory activity in RR multiple sclerosis patients is not fully and rapidly associated with a similar effect on the global neurodegenerative processes. This study also suggests that any effect of GA in preventing brain volume decrease is not evident early following institution of treatment.

Keywords: multiple sclerosis; brain volume; MRI; glatiramer acetate; clinical trial

Abbreviations: ANCOVA = analysis of covariance; EDSS = Expanded Disability Status Scale; GA = glatiramer acetate; Gd = gadolinium; RR = relapsing–remitting

Introduction

A decrease of parenchymal volume leading to global brain atrophy is frequent in patients with multiple sclerosis (Jagust and Noseworthy, 2000). This is considered a consequence of diffuse demyelination and axonal loss (Raine, 1997), which occur as the result of damage in T₂-visible lesions and the normal-appearing white matter (Allen and McKeown, 1979). The correlation between multiple sclerosis inflammatory activity and the development of brain atrophy has not been

fully elucidated. However, increasing evidence indicates that these two phenomena may be, at least partially, independent and evolve with different patterns in the various disease phenotypes (Coles *et al.*, 1999; Simon *et al.*, 1999; Filippi *et al.*, 2000; Ge *et al.*, 2000b; Paolillo *et al.*, 2000).

Cross-sectional and longitudinal studies with MRI (Losseff *et al.*, 1996; Dastidar *et al.*, 1999; Edwards *et al.*, 1999; Liu *et al.*, 1999; Rudick *et al.*, 1999; Simon *et al.*, 1999;

Stevenson *et al.*, 1999; Fox *et al.*, 2000; Ge *et al.*, 2000b; Paolillo *et al.*, 2000; Wolinsky *et al.*, 2000) have investigated the magnitude of the correlation between brain volume and multiple sclerosis clinical findings. These studies showed that brain atrophy can develop in the early, relapsing–remitting (RR) phases of the disease (Rudick *et al.*, 1999; Simon *et al.*, 1999; Ge *et al.*, 2000b; Paolillo *et al.*, 2000). The amount of tissue loss is, however, more pronounced in patients with more disabling, chronic progressive disease courses (Losseff *et al.*, 1996; Dastidar *et al.*, 1999; Stevenson *et al.*, 1999; Ge *et al.*, 2000b; Wolinsky *et al.*, 2000). Since conventional MRI measures lack pathological specificity and are only modestly correlated with disability (Rovaris and Filippi, 1999), the measurement of brain volume has been claimed as an objective marker of multiple sclerosis severity with the potential to accurately monitor disease evolution (Jagust and Noseworthy, 2000). For these reasons, recent clinical trials (Paolillo *et al.*, 1999; Rudick *et al.*, 1999; Filippi *et al.*, 2000; Molyneux *et al.*, 2000) included brain volume measurements as an additional exploratory measure of outcome.

Glatiramer acetate (GA; Copaxone®; TEVA Pharmaceutical Industries Ltd, Petah Tiqua, Israel) is an immunomodulating drug currently approved in several countries for the treatment of RR multiple sclerosis (Johnson *et al.*, 1995, 1998). GA is the acetate salt of a mixture of synthetic polypeptides and it appears to act against multiple sclerosis via production of specific T-suppressor cells that cross react with myelin basic protein in the central nervous system (Aharoni *et al.*, 1999, 2000). On stimulation, these cells secrete regulatory cytokines of the type that characterize Th2 or regulatory T cells (Duda *et al.*, 2000; Neuhaus *et al.*, 2000). Clinically, GA significantly reduces the frequency of relapses in RR multiple sclerosis (Johnson *et al.*, 1995, 1998). In addition, a recent multicentre, placebo-controlled study (Comi *et al.*, 2001) has demonstrated that GA is also effective in reducing multiple sclerosis activity and accumulated burden of disease as measured by serial MRI scans of the brain.

Only a pilot study of a small subcohort of patients participating in the pivotal US trial (Ge *et al.*, 2000a) addressed the issue of the effect of GA treatment on MRI-measured brain volume change. The study showed that the rate of brain volume decrease was significantly higher in placebo than in treated patients over a 2-year follow-up. In addition, a cross-sectional analysis of data from the extended, open-label follow-up of the same trial (Wolinsky *et al.*, 2001) seems to indicate that long-term treatment with GA might prevent the loss of brain parenchyma in RR multiple sclerosis patients.

The present study probed: (i) the extent, if any, of the effect of GA treatment on brain atrophy in RR multiple sclerosis; (ii) the correlations between the effects of GA on different MRI markers reflecting several pathological aspects of multiple sclerosis; and (iii) the relationship of brain atrophy with previous and concomitant inflammatory activity. Data from the European/Canadian multiple sclerosis/MRI

Copaxone Trial (Comi *et al.*, 2001) were analysed for these purposes.

Material and methods

Patients

All patients had to have an age of 18–50 years inclusive, a diagnosis of clinically definite multiple sclerosis (Poser *et al.*, 1983) for at least 1 year, an RR disease course (Lublin *et al.*, 1996), an Expanded Disability Status Scale (EDSS) score (Kurtzke, 1983) of 0.0–5.0, at least one documented relapse in the preceding 2 years and at least one gadolinium (Gd)-enhancing lesion on their screening brain MRI. Patients had to be clinically relapse-free and without steroid treatment in the 30 days prior to the inclusion into the study. Additional information about the inclusion and exclusion criteria is extensively reported elsewhere (Comi *et al.*, 2001). The ethical committees of all participating centres approved the study protocol and each patient signed a written informed consent prior to trial entry.

Study design

The study consisted of a 1-month screening phase, followed by two treatment phases, each lasting 9 months. For trial purposes, a month was defined as of 4-week duration (28 ± 7 days). The first treatment phase was randomized, double-blind and placebo-controlled. The second was an open-label phase, during which all patients received active treatment. Treatment consisted of daily administration of 20 mg GA or placebo by subcutaneous injection. All patients underwent physical and neurological examination, including EDSS rating, laboratory studies and brain MRI at screening, baseline, every month during the double-blind phase and every 3 months during the open-label phase (Comi *et al.*, 2001).

MRI acquisition

The imaging protocol consisted of dual echo, pre- and post-contrast (0.1 mmol/kg Gd) T₁-weighted SE (spin echo) images of the brain (Comi *et al.*, 2001). For brain volume measurements, unenhanced T₁-weighted images obtained at baseline (i.e. when patients were randomized), the end of double-blind phase (i.e. month 9) and the end of open-label phase (i.e. month 18) were analysed. Acquisition parameters for these images were: TR (repetition time) = 450–650 ms; TE (echo time) = 10–20 ms; slices = 44, contiguous, 3 mm thick; in-plane resolution = $\sim 1 \times 1$ mm; signal averages = 2. MRI parameters and scanner were always the same for any given patient for the whole study duration.

MRI analysis

The identification of hyperintense T₂ lesions on the dual echo images, and of Gd-enhancing and hypointense lesions on the

post-contrast T₁-weighted images, was done by consensus of two experienced observers. On the follow-up scans, new Gd-enhancing and new hyperintense T₂ lesions were also counted. Total T₂ hyperintense, T₁ Gd-enhancing and T₁ hypointense lesion volumes were then calculated using a semi-automated local thresholding technique for lesion segmentation and marked hardcopies as a reference as previously detailed (Comi *et al.*, 2001).

Measurements of brain volumes were done using a seed-growing technique for brain parenchyma segmentation from T₁-weighted images, which is fully described elsewhere (Rovaris *et al.*, 2000). The volume was calculated for a slab of brain tissue including the seven contiguous slices rostral to the velum interpositum. A single observer chose the slices to be included in the measurements on the basis of standard neuroanatomical landmarks. Comparison with subsequent scans from each individual patient allowed consistent slice choice and minimized the effects of volume variation due to patient positioning on serial scans. All volume measurements were then done by another observer, who was unaware of the acquisition order of the scans and to whom the scans belonged. Such an approach, which includes the regions where multiple sclerosis pathology is more frequent, has proved to be as sensitive to multiple sclerosis-related changes as measures of the whole of the brain tissue volume (Rovaris *et al.*, 2000). This approach is also highly reproducible, with a mean intra-observer coefficient of variation for repeated measurements of 1.5% (Rovaris *et al.*, 2000).

Statistical analysis

Demographic, clinical and MRI characteristics of subjects in the two study arms were compared using the two-sample *t*-test for the continuous variables and the chi-square test or the Fisher's exact test for the categorical variables. The effect of treatment on brain volume indices was assessed using the analysis of covariance (ANCOVA). This analysis accounted for centre variability and included age, gender, disease duration, brain volume and number of Gd-enhancing lesions at baseline as covariates. ANCOVA was repeated after stratifying patients into those with and without one or more Gd-enhancing lesions on their baseline scans. The significance of the within-group changes during the study periods was analysed using the *t*-test for paired samples. The correlations between brain volume indices and clinical or MRI-derived variables were assessed using the Spearman rank correlation coefficient.

Results

The original trial randomized 119 patients to GA and 120 patients to placebo treatment (Comi *et al.*, 2001). Imaging data for brain volume measurements in this study was available from 113 GA and 114 placebo-randomized subjects that included a baseline, and at least one additional scan at either 9 or 18 months on study. Image data loss was the

result of patient loss from the trial. The reasons for subject drop-outs are listed in Table 1. Table 2 contains the demographic, clinical and MRI characteristics of this brain volume evaluable patient cohort at study entry. These characteristics are similar to those for the original study cohort (Comi *et al.*, 2001) and no significant differences between the two arms were found for any of the tested variables.

Brain volume changes

The absolute values of brain volume and their changes during the study for all the patients are reported in Table 3 and graphically displayed in Fig. 1. The average brain volume at study entry was not significantly different between patients in the two study arms. Mean brain volume significantly decreased during the two study phases both in patients originally randomized to GA, and in those originally randomized to placebo. During the double-blind phase, an average brain volume reduction of 0.7 and 0.8% was seen in placebo and GA-treated patients, respectively. The rate of brain volume decrease was lower during the open-label phase for the subjects that had been on continuous GA treatment from randomization (0.6% loss for those originally on placebo, 0.4% for those always on GA), but these differences were not significant. After 18 months, brain volume was decreased by 1.4 and 1.2% in patients originally randomized to placebo and GA treatment, respectively. Neither the absolute nor the percentage changes of brain volume were significantly different between the two study arms. No significant differences between the two study arms were found when the analysis was repeated after stratifying patients into those with and those without one or more Gd-enhancing lesions on their baseline scan (data not shown).

Covariate analysis was performed that included age, gender, centre, disease duration and brain volume at baseline. No significant effect of treatment was found on the absolute or percentage change of brain volume during the double-blind phase of the study (ANCOVA; *P* = 0.92 and 0.88, respectively). No significant treatment effect on the absolute and percentage change of brain volume was found (*P* = 0.92 and 0.87, respectively) when ANCOVA was repeated after adding the number of Gd-enhancing lesions at baseline to the covariates.

Correlations between brain volume and other MRI-derived measures

The correlations between MRI measures of disease activity or burden at baseline and brain volume change during the study periods are summarized in Table 4. The number of Gd-enhancing lesions on baseline scans was moderately, but significantly, correlated with the absolute brain volume changes during the double-blind phase of the study in the whole patient cohort and in placebo-treated patients. This

Table 1 Drop-outs from the original trial cohort of 239 patients at the individual scan time-points

Reasons for drop-outs	Treatment group			
	Original placebo		Original GA	
	M9	M18	M9	M18
Adverse experiences	2	6	2	4
Patient refusal to continue MRI monitoring	2	2	–	–
Pregnancy	–	1	–	–
Consent withdrawn for patient decision	–	1	4	5
Lost to follow-up	1	1	–	–
Other	1	1	–	1
All	6	12	6	10

M9 = month 9 scan; M18 = month 18 scan.

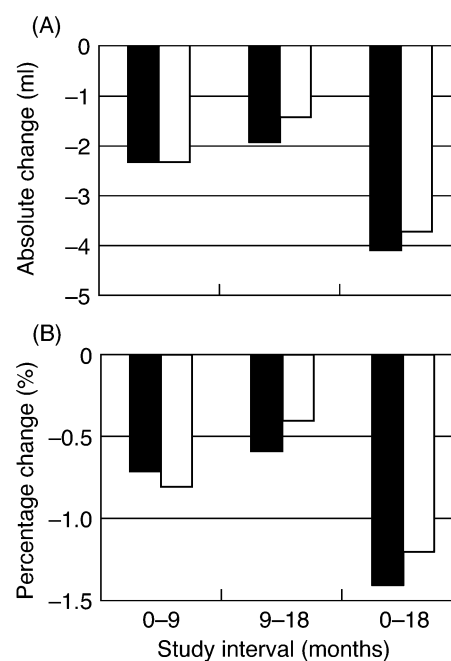
Table 2 Baseline clinical and MRI characteristics (mean \pm standard deviation) of study subjects

	Original placebo (n = 114)	Original GA (n = 113)
Gender (female/male)	83/31	87/26
Age (years)	34.0 \pm 7.6	34.4 \pm 7.4
Disease duration (months)	99.6 \pm 67.0	96.0 \pm 66.6
Prior 2-year relapse rate	2.4 \pm 1.4	2.7 \pm 1.7
EDSS score	2.4 \pm 1.2	2.3 \pm 1.1
Gd-enhancing lesion number	4.5 \pm 7.2	4.1 \pm 4.7
Gd-enhancing lesion volume (ml)	0.7 \pm 2.3	0.5 \pm 0.7
T ₂ hyperintense lesion volume (ml)	20.8 \pm 19.0	18.9 \pm 15.5
T ₁ hypointense lesion volume (ml)	4.1 \pm 5.0	3.3 \pm 3.9

For statistical analysis, see the text.

correlation was not significant in the GA-treated patients, nor was there any significant correlation between enhancing lesion burden at baseline and brain volume changes during the open-label phase of the study. The total volumes of hyperintense T₂ and hypointense T₁ lesions on baseline scans were significantly correlated with baseline brain volumes. In placebo-treated patients, baseline lesion volumes were also significantly correlated with the absolute brain volume change during the double-blind phase of the study. The magnitude of these correlations was similar when percentage instead of absolute change of brain volume was considered (data not shown).

Table 5 reports the correlations between MRI measures of disease activity or accumulated disease burden during the double-blind phase of the study and brain volume change. The numbers of Gd-enhancing lesions and new T₂ hyperintense lesions detected from monthly scans obtained during the double-blind phase of the study were moderately, but significantly, correlated with the absolute brain volume changes during the same period in the placebo-treated, but not in the GA-treated patients. No significant correlations were found between MRI activity during the double-blind phase and loss of brain volume during the open-label phase of the study. Changes in hypointense T₁ lesion volume during

**Fig. 1** Mean absolute (A) and percentage (B) changes of brain volume in patients treated with placebo (black bars) and GA (open bars) during the double-blind phase of the study.

the double-blind phase were significantly correlated with brain volumes on baseline scans for both placebo and GA-treated patients. On the contrary, hyperintense T₂ lesion volume changes were significantly correlated with brain volumes at baseline only in patients treated with GA for the entire study duration. The magnitude of the reported correlations was similar when percentage instead of absolute change of brain volume was considered (data not shown).

Correlations between brain volume and clinical variables

In the entire cohort, brain volume was modestly, but significantly correlated with EDSS score at baseline ($r =$

Table 3 Absolute values and changes of patients' brain volume during the two study phases

	Original placebo	Original GA
Brain volume: baseline (ml)		
Mean	300.5	302.7
SD	23.9	23.2
Range	232.5–373.4	252.6–356.0
n	114	113
Brain volume: month 9 (ml)		
Mean	298.3	300.4
SD	24.1	23.7
Range	229.0–366.6	247.8–358.9
n	114	113
Brain volume: month 18 (ml)		
Mean	297.0	299.3
SD	24.2	23.3
Range	223.2–357.0	247.1–354.0
n	108	109
Change from baseline to month 9 (ml)		
Mean	–2.3	–2.3
SD	6.5	5.8
Range	21.6–24.2	28.8–11.4
P value	0.005	0.0001
Change from baseline to month 9 (%)		
Mean	–0.7	–0.8
SD	2.2	1.9
Range	–7.4–9.4	–8.5–3.6
P value	0.0029	0.0001
Change from month 9 to month 18 (ml)		
Mean	–1.9	–1.4
SD	6.1	5.1
Range	–19.5–11.5	–18.7–9.2
P value	0.0021	0.0067
Change from month 9 to month 18 (%)		
Mean	–0.6	–0.4
SD	2.0	1.7
Range	–6.9–4.4	–5.9–2.9
P value	0.0027	0.0088
Change from baseline to month 18 (ml)		
Mean	–4.1	–3.7
SD	7.0	7.5
Range	–26.1–17.0	–39.4–17.6
P value	0.001	0.0001
Change from baseline to month 18 (%)		
Mean	–1.4	–1.2
SD	2.3	2.4
Range	–7.7–5.4	–11.6–5.4
P value	0.0001	0.0001

–0.22, $P = 0.0012$), month 9 ($r = -0.26$, $P = 0.0003$) and month 18 ($r = -0.19$, $P = 0.0065$). At baseline, this correlation was stronger in patients randomized to GA treatment ($r = -0.30$, $P = 0.0013$) than in those randomized to placebo treatment ($r = -0.13$, $P = 0.16$). However, its magnitude did not differ between the two arms at month 9 ($r = -0.26$). At month 18, the correlation between brain volume and EDSS score was stronger in patients originally

randomized to placebo ($r = -0.25$, $P = 0.01$) than in those originally randomized to GA treatment ($r = -0.13$, $P = 0.20$). No significant correlations were found between brain volume at baseline and frequency of relapses in the two years prior to study entry.

During the double-blind phase of the study, patients treated with GA or placebo had a mean EDSS change of -0.04 and $+0.01$, and the mean frequency of clinical relapses in the two arms was 0.54 and 0.72, respectively. No significant correlations were found between brain volume at baseline or month 9 and EDSS change or frequency of clinical relapse either in GA or in placebo-treated patients. The frequency of clinical relapses during the first 9 months was not significantly correlated with the decrease of brain volume during the subsequent phase of the study. Brain volume changes and EDSS changes were not significantly correlated during either of the study phases.

All the group and correlation analyses were repeated after excluding patients who underwent month 9 and/or month 18 scans while on steroid treatment (nine and six patients, respectively). None of the results changed significantly (data not shown).

Discussion

Several recent placebo-controlled trials (Paolillo *et al.*, 1999; Rudick *et al.*, 1999; Filippi *et al.*, 2000; Molyneux *et al.*, 2000) consistently showed that any effect of treatment in reducing the rate at which brain atrophy develops in multiple sclerosis patients was moderate at best, even though the same treatments were highly effective in favourably modifying other MRI measures of multiple sclerosis activity and burden. These findings, and the results of the present investigation, prompt speculation on the correlation between various aspects of multiple sclerosis pathology, and on the impact that available treatments proven to be effective in reducing multiple sclerosis-related inflammation may have on the most severe and disabling pathological substrates of the disease.

All multiple sclerosis patients participating in the present study had to have MRI evidence of ongoing inflammation at study entry (Comi *et al.*, 2001). Therefore, the average extent of MRI-monitored multiple sclerosis activity during the first 9 months was high and enabled us to investigate the correlations between brain volume change and other MRI markers of multiple sclerosis activity and burden. T_2 lesion load is an overall measure of accumulated disease burden that includes all of the heterogeneous substrates of multiple sclerosis lesions. T_1 hypointense lesion load has a higher pathological specificity, reflecting areas of severe tissue disruption and axonal damage (van Walderveen *et al.*, 1998). We found that both T_2 and T_1 lesion volumes at baseline were modestly correlated with brain volume at baseline in both treatment groups. In addition, during the first 9 months the rate of brain volume loss in placebo-treated patients modestly correlated with the number of Gd-enhancing lesions and the volumes of Gd-enhancing, T_2 -hyperintense and T_1 -

Table 4 Correlations between MRI characteristics at baseline and baseline values or changes of brain volume during the two study periods

	All patients				Original placebo				Original GA			
	BV	BVD 9–0	BVD 18–9	BVD 18–0	BV	BVD 9–0	BVD 18–9	BVD 18–0	BV	BVD 9–0	BVD 18–9	BVD 18–0
EL	0.05	-0.28 (0.0001)	-0.02	-0.25 (0.0002)	0.08	-0.34 (0.0002)	-0.01	-0.33 (0.0002)	-0.01	-0.16	-0.02	-0.14
ELV	0.01	-0.24 (0.0003)	-0.03	-0.22 (0.001)	0.01	-0.31 (0.0008)	-0.03	-0.31 (0.0009)	0.03	-0.09	-0.04	-0.09
T ₂ LV	-0.24 (0.0002)	-0.18 (0.008)	0.04	-0.11	-0.21 (0.03)	-0.41 (0.0001)	0.11	-0.27 (0.004)	-0.28 (0.002)	0.14	-0.07	0.08
T ₁ LV	-0.26 (0.0001)	-0.11	0.03	-0.07	-0.23 (0.01)	-0.32 (0.0005)	0.08	-0.23 (0.02)	-0.30 (0.001)	0.19	-0.05	0.13

Values are Pearson correlation coefficients (significant correlations are in bold; *P* values in brackets). BV = brain volume on baseline scans; BVD 9–0, BVD 18–9 and BVD 18–0 = absolute differences in brain volume between month 9 and baseline, month 18 and month 9, month 18 and baseline scans, respectively; EL = number of Gd-enhancing lesions on baseline scans; ELV = volume of Gd-enhancing lesions on baseline scans; T₂ LV = volume of T₂ hyperintense lesions on baseline scans; T₁ LV = volume of T₁ hypointense lesions on baseline scans.

Table 5 Correlations between measures of MRI activity or accumulated disease burden and baseline values or changes of brain volume during the two study periods

	All patients				Original placebo				Original GA			
	BV	BVD 9–0	BVD 18–9	BVD 18–0	BV	BVD 9–0	BVD 18–9	BVD 18–0	BV	BVD 9–0	BVD 18–9	BVD 18–0
TEL	0.07	-0.25 (0.0001)	0.01	-0.21 (0.002)	0.12	-0.30 (0.001)	0.02	-0.26 (0.007)	0.01	-0.18	-0.01	-0.15
NEL	0.05	-0.25 (0.0001)	-0.01	-0.21 (0.001)	0.12	-0.31 (0.0008)	0.02	-0.27 (0.005)	-0.04	-0.18	-0.02	-0.16
NT ₂ L	0.11	-0.24 (0.0003)	-0.01	-0.21 (0.002)	0.15	-0.26 (0.005)	-0.01	-0.25 (0.01)	0.09	-0.22 (0.02)	0.01	-0.17
T ₂ DLV	-0.10	0.07	0.10	0.14	-0.03	0.10	0.08	0.17	-0.26 (0.005)	0.01	0.19 (0.04)	0.13
T ₁ DLV	-0.21 (0.001)	-0.09	0.07	-0.01	-0.19 (0.04)	-0.17	0.12	-0.03	-0.24 (0.01)	0.03	0.01	0.03

Values are Pearson correlation coefficients (significant correlations are in bold; *P* values in brackets). BV = brain volume on baseline scans; BVD 9–0, BVD 18–9 and BVD 18–0 = absolute differences in brain volume between month 9 and baseline, month 18 and month 9, month 18 and baseline scans, respectively; TEL = total number of Gd-enhancing lesions on monthly scans from baseline to month 9; NEL = number of new Gd-enhancing lesions on monthly scans from baseline to month 9; NT₂L = number of new T₂ hyperintense lesions on monthly scans from baseline to month 9; T₂ DLV = absolute difference in T₂ hyperintense lesion volume between baseline and month 9 scans; T₁ DLV = absolute difference in T₁ hypointense lesion volume between baseline and month 9 scans.

hypointense lesions on their baseline scans. The number of active lesions on monthly scans during the double-blind phase was significantly correlated with the rate of brain volume loss during the same period for the whole patient cohort and the placebo group, but not for the treated group. This correlation was still significant when brain volume changes over 18 months were considered. Only for patients

treated with GA was the increase of T₂ lesion volume during the double-blind phase of the study significantly correlated with baseline brain volume. Hypointense T₁ lesion volume change during the first 9 months had a significant, but modest correlation with baseline brain volumes, but not with the rate of brain volume loss during either study phase.

The results of this study indicate that the rate of brain

volume loss in RR multiple sclerosis patients over 18 months is weakly, but significantly, influenced by previous and ongoing disease activity. While, in GA-treated patients, the lack of a correlation between changes of brain volume and other MRI measures can be due to the effect of treatment in reducing both lesion activity and accumulation (Mancardi *et al.*, 1998; Comi *et al.*, 2001), data from patients treated with placebo during the double-blind phase suggest that mechanisms other than inflammation, including failure of remyelination, loss of trophic factors (Kaplan *et al.*, 1997), disturbances of electrical conduction (Pfrieger and Barres, 1997) or long-term sublethal axonal injury in persistently demyelinated fibres might account for the progressive axonal loss observed in multiple sclerosis (Trapp *et al.*, 1999). Some reparative mechanisms following multiple sclerosis injury, such as remyelination and axonal sprouting, can occur over relatively long periods. Therefore, in patients with early and active RR multiple sclerosis, the effects of these reparative processes in preventing cerebral atrophy might only be apparent late, after a prolonged reduction in the inflammatory activity is well established. Two other factors might, however, contribute to the lack of correlation between baseline MRI-measured multiple sclerosis burden of disease and subsequent brain atrophy development in GA-treated patients. On the one hand, the reduction of inflammatory activity may lead to an apparent decrease of cerebral volume. On the other, a real increase of brain atrophy might occur as a consequence of a diminution of the beneficial effect of inflammation on tissue repair. It is also quite conceivable that the rate of brain volume loss from a given time point on may depend upon the amount of prior inflammatory activity. This is supported by data from a crossover design trial of Campath 1H in secondary progressive multiple sclerosis patients (Coles *et al.*, 1999; Paolillo *et al.*, 1999). Despite almost complete suppression of Gd-enhancement, brain atrophy progressed in patients experiencing clinical deterioration at a rate that was highly correlated ($r = 0.77$) with the frequency of Gd-enhancing lesions during the pre-Campath 1H treatment period.

During the double-blind phase of the study, GA treatment did not have any measurable impact on the decrease of brain volume, which was of similar magnitude in both study arms. The subsequent open-label phase of the study did not show any significant change in the rate of brain volume loss. Over 18 months, the average annual decrease in brain volume was ~1% in the entire trial population, confirming that progressive brain volume loss occurs in RR multiple sclerosis patients over relatively short intervals. This finding is consistent with previous studies (Rudick *et al.*, 1999; Ge *et al.*, 2000a, b), where whole brain volume decreased by 0.5–1.8% yearly in untreated RR multiple sclerosis patients.

Several factors might explain why, in this study, the effect of GA in reducing clinical and MRI-measured multiple sclerosis activity (Comi *et al.*, 2001) was not paralleled by an effect against the observed decrease of patients' brain volume. These include: (i) the short duration of the follow-

up; (ii) the mechanisms of action of GA; and (iii) the persistence of low-grade inflammation undetected by conventional MRI techniques that could cause progressive tissue loss.

Undoubtedly the short duration of the placebo-controlled phase limited our ability to detect any effect of GA treatment on brain volume change. In this patient group some effect of GA on the MRI measures was discernible 2 months after initiating treatment and the magnitude of the effect of active treatment increased over time. However, the effects of GA on relapses and on other MRI measures of multiple sclerosis activity became significant only after 6 months of treatment (Comi *et al.*, 2001). These findings fit well with the timing of drug action in the modulation of T-cell immune responses (Neuhaus *et al.*, 2000). Thus, if the effect of GA treatment in slowing the loss of brain volume in multiple sclerosis is similarly delayed, it may not be surprising that no effect was evident over the 9-month placebo-controlled comparison. The small differences observed between the groups during the second 9 months of observation in the rates of brain volume loss, while not significant, could be predictive of treatment effects on longer follow-up. Similarly, Rudick and colleagues were only able to detect a significant slowing of brain volume loss in RR multiple sclerosis patients treated with interferon beta-1a during the second year of treatment, despite the rapid suppression of MRI inflammatory activity that can be achieved using this drug (Rudick *et al.*, 1999; Waubant *et al.*, 1999). Interestingly, we also found that the average rate of brain volume decrease was 50% lower during the second than during the first 9 months of the study (0.4% versus 0.8%, respectively) for patients originally treated with GA, whilst it was similar during the two study phases for patients originally treated with the placebo.

A second explanation for our findings might be the limited ability of GA to modify the pathological mechanisms leading to global tissue loss in multiple sclerosis. A similar apparent divergence between the anti-inflammatory effects and the prevention of brain volume reduction over time was reported for interferon beta-1b (Molyneux *et al.*, 2000), cladribine (Filippi *et al.*, 2000) and Campath 1H (Coles *et al.*, 1999; Paolillo *et al.*, 1999). The modest magnitude of the correlation between Gd-enhancement and brain tissue loss also supports the hypothesis that the impact of treatment on MRI measures closely related to inflammatory activity may not necessarily be rapidly or fully translated into a beneficial effect on other MRI measures which reflect tissue loss. On the other hand, pathological studies (Trapp *et al.*, 1998) have shown that axonal damage occurs in inflammatory multiple sclerosis lesions, thus suggesting that preventing brain inflammation should have at least a partial effect on the progressive loss of tissue seen in multiple sclerosis patients. The magnitude of this effect might, however, be too small to be detected by measurements of brain tissue volume over relatively short intervals.

The inability of GA to prevent short-term brain tissue loss in RR multiple sclerosis might also be due to the persistence

of low-grade inflammatory activity that could go undetected with standard MRI. That some degree of inflammatory activity could still occur in GA-treated patients is also suggested by the observation that the relapse rate is only partially reduced (Comi *et al.*, 2001). Whether biologically important inflammatory activity persists in the brains of patients undergoing immunomodulatory treatments that can only be detected with increased sensitivity Gd-enhanced MRI methods (Silver *et al.*, 1997; Filippi *et al.*, 1998a, b) is an unresolved issue. Preliminary data, however, suggest that this is not the case for patients treated with low doses of interferon beta-1a (Rovaris *et al.*, 1999), since the amount of activity detected by triple dose Gd-enhanced MRI was found to be lower under treatment than during a pre-treatment screening phase. It is also uncertain whether, and to what extent, mild degrees of brain inflammation might contribute to the development of brain atrophy.

In conclusion, we were unable to find a significant effect of GA on reducing the brain tissue loss that occurs in RR multiple sclerosis over 9 months. However, the results of this study have several important implications. First, they confirm that assessment of brain volume on serial MRI provides a reliable measure of brain atrophy, an important component of the pathology of multiple sclerosis. Among RR multiple sclerosis patients selected for high clinical and MRI disease activity, the loss of cerebral volume can be significant even over relatively short intervals, indicating that tissue loss including the loss of myelin and axons occurs even during the earliest, non-disabling phases of multiple sclerosis. Secondly, they indicate that multiple sclerosis inflammatory activity is only in part responsible for the development of brain atrophy. Thirdly, these results strengthen recent evidence that immunomodulating and immunosuppressive treatments that reduce multiple sclerosis inflammatory activity and lesion accumulation may not be translated into a similar effect on progressive tissue loss, either in RR or progressive multiple sclerosis patients. Fourthly, they indicate that brain volume measures might be used to test the efficacy of experimental multiple sclerosis treatments targeted to non-inflammatory components of the disease process. However, such studies must be of considerable duration to assess adequately the efficacy of any treatment on multiple sclerosis-related global cerebral tissue loss.

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