European/Canadian Multicenter, Double-Blind, Randomized, Placebo-Controlled Study of the Effects of Glatiramer Acetate on Magnetic Resonance Imaging–Measured Disease Activity and Burden in Patients with Relapsing Multiple Sclerosis

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Two prior double-blind, placebo-controlled, randomized trials demonstrated that glatiramer acetate (GA) reduces relapse rates in patients with relapsing remitting multiple sclerosis (RRMS). This study was designed to determine the effect, onset, and durability of any effect of GA on disease activity monitored with magnetic resonance imaging (MRI) in patients with RRMS. Two hundred thirty-nine eligible patients were randomized to receive either 20 mg GA ($n = 119$) or placebo ($n = 120$) by daily subcutaneous injection. Eligibility required one or more relapses in the 2 years before entry and at least one enhancing lesion on a screening MRI. The study was a randomized, double-blind, placebo-controlled phase during which all patients studied underwent monthly MRI scans and clinical assessments over 9 months. The primary outcome measure was the total number of enhancing lesions on T1-weighted images. Secondary outcome measures included the proportion of patients with enhancing lesions, the number of new enhancing lesions and change in their volume; the number of new lesions detected on T2-weighted images and change in their volume, and the change in volume of hypointense lesions seen on unenhanced T1-weighted images. Clinical measures of disease activity were also evaluated. The active treatment and placebo groups were comparable at entry for all demographic, clinical, and MRI variables. Treatment with GA showed a significant reduction in the total number of enhancing lesions compared with placebo (−10.8, 95% confidence interval −18.0 to −3.7; $p = 0.003$). Consistent differences favoring treatment with GA were seen for almost all secondary end points examined: number of new enhancing lesions ($p < 0.003$), monthly change in the volume of enhancing lesions ($p = 0.01$), and change in volume ($p = 0.006$) and number of new lesions seen on T2-weighted images ($p < 0.003$). The relapse rate was also significantly reduced by 33% for GA-treated patients ($p = 0.012$). All effects increased over time. Glatiramer acetate significantly reduced MRI-measured disease activity and burden.

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) affecting about 1 million people worldwide. The disease leads to substantial disability in most patients.[1] Converging evidence supports the concept that MS is an immune-mediated disease of genetically susceptible subjects that is unleashed by one or more environmental agents. A myelin antigen-specific, Th1-type T-cell–orchestrated immunopathogenesis directed at one or more immunodominant antigenic fragments of myelin, including myelin basic protein (MBP), proteolipid protein (PLP), and/or oligodendrocyte glycoprotein (MOG), is postulated to be central to the immune dysfunction that underlies the disorder.[2]

Prior therapeutic strategies to modify the course of MS, based on nonselective immunosuppression to reduce or eliminate the offending T cells, have been of limited benefit and are often associated with significant

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side effects. Recently, several classes of immunomodulatory agents have been evaluated,[3] and several formulations of recombinant interferon-β are now approved for the treatment of relapsing-remitting (RR) MS. The only other immunomodulator currently approved in several countries for the treatment of RRMS is glatiramer acetate (GA) (Copaxone®, formerly known as copolymer 1).[4–6]

Glatiramer acetate was developed for human use based on its effectiveness in preventing or reducing the severity of neurological disease in experimental allergic encephalomyelitis (EAE).[7] Previous clinical trials showed that GA reduces relapse rate and the accumulation of disability in patients with RRMS.[4–6] Although perivascular inflammation is a prominent element of the pathology of MS lesions that can be monitored using enhanced magnetic resonance imaging (MRI),[8,9] limited data are available to assess the effect of GA on this and other MRI-defined aspects of the disease.[10,11]

The present study was a placebo-controlled, double-blind clinical trial to determine the effect of GA on the cumulative number of enhancing lesions found on monthly MRI, and to assess other MRI measures that might be affected by treatment in a large cohort of patients with RRMS. This study was designed to determine whether treatment with GA is associated with a measurable effect on the inflammatory aspect of the disease, and to define the time course of the evolution of any effect.

Patients and Methods

Patients

Twenty-nine centers in six European countries and Canada participated in the trial. Enrollment started in February 1997 and concluded in November 1997. Of 485 patients screened with clinically definite MS,[12] 252 met all entry criteria and 239 of these were randomized to treatment with placebo or GA, 20 mg daily by subcutaneous injection. Thirteen patients were excluded at the entry visit based on the occurrence of a relapse, steroid treatment, or abnormalities found on one or more screening tests. The clinical entry and exclusion criteria were nearly identical to those in the pivotal American trial.[5] All patients had to have an RR course, a diagnosis of MS for at least 1 year, an age of 18 to 50 years inclusive, a Kurtzke Expanded Disability Status Scale (EDSS)[13] score of 0 to 5, at least one documented relapse in the preceding 2 years, and at least one enhancing lesion on their screening brain MRI. Of note, prior controlled pivotal trials of GA in RRMS did not use MRI and required a minimum of two clinical attacks in the 2 years before enrollment.[4–6] Patients had to be clinically relapse-free and without steroid treatment in the 30 days before their pre-entry MRI. The previous use of GA or oral myelin led to exclusion, as did prior lymphoid irradiation, the use of immunosuppressant or cytotoxic agents in the past 2 years, or the use of azathioprine, cyclosporine, interferons, deoxyspergualine, or chronic corticosteroids during the previous 6 months. Subjects receiving concomitant therapy with an experimental drug for MS or for another disease were ineligible. Patients with other serious intercurrent systemic or psychiatric illnesses, and those who were pregnant or unwilling to practice reliable methods of contraception during the course of the study were not enrolled. Patients with known hypersensitivity to gadolinium-DTPA (Gd) or those unable to undergo repeated MRI studies were excluded. The ethical committees of all participating centers approved the study.

Design

The study was a double-blind, placebo-controlled, randomized study lasting 9 months. For trial purposes a month was defined as 4 weeks (28 ± 7 days). At the pre-enrollment visit eligible patients were informed about all aspects of the study and gave written informed consent. Eligible patients underwent physical and neurological examination, including EDSS and Ambulation Index,[14] laboratory studies, and brain MRI. The coordinating center reviewed the results of the pre-enrollment evaluations including the brain MRI, and when all inclusion and exclusion criteria were satisfied, gave the approval for patient enrollment. Patients were randomized to receive 20 mg GA or placebo daily by subcutaneous injection.

The randomization list, stratified by individual study center, was computer-generated by the TEVA Statistical Data Management Department. Equal allocation of the two treatment groups was used. At each study site a treating neurologist was responsible for the overall medical management of the patient including safety monitoring. Vital signs and adverse effects were assessed monthly. An examining neurologist was responsible for all scheduled neurological examinations and exacerbation follow-up. All patients had neurological evaluation every 3 months; additional assessments were carried out for symptoms suggestive of a relapse. MRI was performed on all patients at monthly intervals. Safety evaluations that included vital signs, hematology, and biochemical tests were performed every 3 months at all regularly scheduled clinical visits. All personnel involved in the study were unaware of the treatment allocation. Patient and physician blinding were not formally assessed because the primary and secondary outcome measures were MRI parameters. Nevertheless, both the treating neurologist and the patient were informed of the importance of not discussing safety issues with the examining neurologist.

A relapse was defined as the appearance of one or more new neurological symptoms, or the reappearance of one or more previously experienced ones. Patients were instructed to telephone their local center immediately if they perceived that they might be experiencing a relapse. A visit was arranged within 7 days of notification. Neurological deterioration had to last at least 48 hours and be preceded by a relatively stable or improving neurological state in the prior 30 days. An event was counted as a relapse only when the patient's symptoms were accompanied by objective changes in the neurological examination corresponding to an increase of at least 0.5 points on the EDSS, or one grade in the score of two or more Functional Systems (FS), or two grades in one FS. Deterioration associated with fever or infection that can cause transient, secondary impairment of neurological func-

tion in MS patients were not considered relapses. Nor was a change in bowel, bladder, or cognitive function alone accepted as a relapse. The principal investigator reviewed all exacerbation reports to check their consistency with this relapse definition. Relapses could be treated with a standard dose of 1.0 g intravenous methylprednisolone for 3 consecutive days.

Outcome Measures

The primary outcome measure was the total number of enhancing lesions. Secondary outcome measures included the total volume of enhancing lesions, proportion of patients with enhancing lesions, number of new enhancing lesions, number of new lesions on T2-weighted images, and percent change of lesion volume on T2-weighted images. The change in the volume of hypointense lesions on T1-weighted images was the final prespecified secondary outcome measure. Relapse rate and other clinical outcomes were tertiary outcomes.

MRI Scanning and Analysis

Before any participating center could enter patients into the trial, they were required to image a volunteer patient with clinically definite MS according to strict study imaging protocol. The images were sent to the Neuroimaging Research Unit in Milan as film and electronic data for review to ensure that high-quality imaging could be performed at each contributing center. Twenty-eight MRI sites were approved (21 scanners were operating at 1.5 T, five at 1.0 T, and two at 0.5 T). Conventional spin-echo sequences (TR 2200–2800, TE 20–50/60–100, 3-mm slice thickness, and 44 contiguous axial slices) were used to obtain proton density and T2-weighted images. Two series of T1-weighted images (TR 450–650, TE 10–20, 3-mm slice thickness, and 44 contiguous axial slices) were obtained before and 5 minutes after the injection, through a long intravenous catheter, of 0.1 mmol/kg of Gd. The slices were positioned to run parallel to a line that joined the most infero-anterior and infero-posterior parts of the corpus callosum. At follow-up, patients were carefully repositioned following published guidelines.[15]

Image quality was reviewed centrally according to predetermined criteria. Unsatisfactory images were rejected, but not repeated except for the 36 weeks scan because of the frequent scanning interval. Patient MRI-based eligibility for study entry was determined by the central image analysis center. Identification of enhancing lesions, high signal intensity lesions on T2-weighted images, and hypointense lesions on T1-weighted unenhanced images was done by consensus of two experienced observers, as previously described.[16,17] Trained technicians then outlined the lesions using a semi-automated segmentation technique based on local thresholding,[18] with reference to the marked hard copies. The lesion volumes were calculated automatically. In a previous study using the same measurement strategy,[18] we showed that the median intraobserver coefficients of variation were 1.6% (range = 1.8% to 6.2%) for T2 and 4.0% (range = 2.2% to 8.4%) for T1 lesion load.

Statistical Analysis

Analyses were based on an intention-to-treat data set. The last observation carried forward (LOCF) method was implemented to account for early discontinuation and missing data. The assessment of data as observed, without carrying forward missing data (AS IS), was also planned to reduce any source of bias in the results due to early withdrawals or missing data. A baseline-adjusted analysis of covariance (ANCOVA) compared the two study arms for the primary end point, incorporating terms for treatment and center as main effects. Covariates were age, gender, baseline EDSS, disease duration, number of relapses in the 2 years before enrollment, and number of enhancing lesions in the prerandomization scan. To better understand the results, analyses were also performed using log transformation, rank transformation with ANCOVA, and quasi-likelihood Poisson regression. Tests for the comparability of study groups at baseline evaluated demographic, clinical, and MRI data. The continuous variables were examined using the two-sample two-sided t test or the Mann–Whitney test when appropriate. The categorical variables were checked for differences between groups using the χ^2 test or Fisher's exact test as appropriate. All p values given are two tailed. Two interim analyses were planned when at least 65 and 130 patients had completed the study. For the first and second interim analyses, treatment effect was considered significant with a p value of ≤ 0.0005 and ≤ 0.014, respectively. The final analysis required a p value of 0.045 or better. Sample size was projected based on literature data and on simulations modeled using a Poisson cyclic variable. We estimated that for an expected treatment effect of 30% or more, a 9-month study with 85 patients in each arm (assuming a 20% dropout rate) would provide more than 85% power to detect a significant difference in the total number of enhancing lesions.

Results

Demographic and Baseline Characteristics

Of the 239 enrolled patients, 119 were randomized to GA and 120 to placebo. Baseline demographic and clinical characteristics did not differ significantly between the two study arms. They were also similar to those of patients who participated in the U.S. pivotal trial of GA and most other clinical trials in RRMS (Table 1). Baseline MRI characteristics were also not different between the two groups.

Study Compliance

Seven patients dropped out in each arm. Seven patients dropped out in the first trimester, five in the second trimester, and two in the third trimester. Two subjects in the placebo group and three in the GA group discontinued treatment because of adverse experiences. One patient in the placebo arm discontinued treatment that he considered ineffective, another left because of poor compliance, one was lost to follow-up, and two refused to continue MRI monitoring. One subject discontinued GA treatment when he moved away from the center, and another after a severe exacerbation. Four GA subjects withdrew their consent without providing a reason. Of 1309 planned MRI sessions in the GA group, 1237 (94.5%) were available for analysis. The comparable proportion in the placebo arm was 96.3%.

Table 1. Baseline Clinical and MRI Characteristics (Mean ± SD) of Trial Subjects and Comparison with Clinical Features of U.S. Pivotal Trial Cohorts

	European/Canadian		U.S. Pivotal	
	Placebo	GA	Placebo	GA
N	120	119	126	125
Age (years)	34.0 ± 7.5	34.1 ± 7.4	34.3 ± 6.5	34.6 ± 6.0
Disease duration (years)	8.3 ± 5.5	7.9 ± 5.5	6.6 ± 5.1	7.3 ± 4.9
Prior 2-year relapse rate	2.5 ± 1.4	2.8 ± 1.8	2.9 ± 1.1	2.9 ± 1.3
EDSS	2.4 ± 1.2	2.3 ± 1.1	2.4 ± 1.3	2.8 ± 1.2
Ambulation index	1.2 ± 1.1	1.1 ± 0.9	1.1 ± 0.9	1.2 ± 1.0
Enhancing lesion number	4.4 ± 7.1	4.2 ± 4.8		
New enhancing lesion number[a]	2.6 ± 4.1	2.5 ± 3.5		
Enhancing lesion volume (ml)	0.7 ± 2.2	0.6 ± 0.7		
New T2 lesion number[a]	1.2 ± 1.7	1.0 ± 1.5		
T2 lesion volume (ml)	20.5 ± 18.8	20.0 ± 17.2		
T1 hypointense lesion volume (ml)	4.0 ± 4.9	3.4 ± 3.9		

[a]New enhancing and T2 lesions at entry were determined by comparing the pre-enrollment scans (obtained to assess whether patients met the MRI criteria for study entry) and the baseline scans. According to protocol, the maximum elapsed interval between these two scans was 28 days. On average, it was 22.2 days for placebo patients and 21.0 days for GA patients.

MRI Outcomes

The mean total number of enhancing lesions was 36.80 for the placebo group and 25.96 for the GA group (Fig 1). The mean reduction in the total number of enhancing lesions in the GA group compared with the placebo group was −10.8 (95% confidence interval [CI], −18.0 to −3.7; $p = 0.003$), a 29% reduction. When analyzed AS IS, the results were similar, reflecting the limited loss of MRI data, with a 35% reduction in the total number of enhancing lesions for the GA treated arm compared with the placebo group (33.7 versus 21.8; $p < 0.001$). The mean number of enhancing lesions per patient per month differed between treatment groups in favor of GA (2.9 versus 4.1; $p < 0.005$). Repeated measures analysis showed a drug interaction with time ($p = 0.027$). Statistically significant differences in the mean number of enhancing lesion per patient per month emerged between the groups at month 6 (Fig 2). The magnitude of the treatment effect seen on the total number of new enhancing lesions paralleled that observed for the total number of enhanced lesions with a 33% reduction for the GA treatment group (17.4 versus 26.0; $p < 0.003$). The time course of the effect of GA on the number of new enhancing lesions was also similar to that on the number of enhancing lesions (data not shown).

The results of secondary MRI end points are sum-

Fig 1. Mean number of cumulative enhancing lesions identified on all postgadolinium T1-weighted images over the 9 months of the study. Data are shown using the last available observation carried forward (LOCF) when MRI data were missing for any specific time interval for a given patient. They are also shown using all available data (As Is) without an adjustment for missing values.

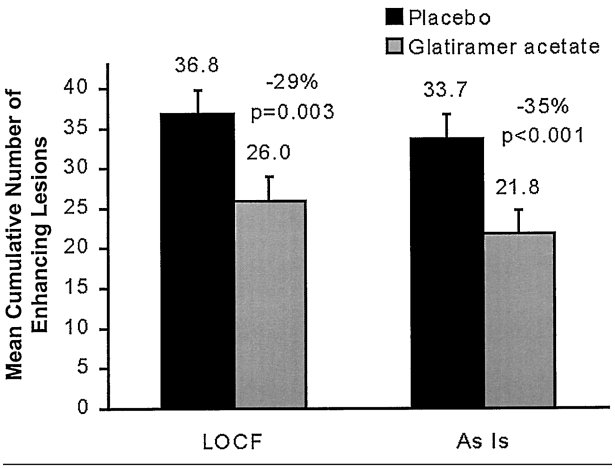
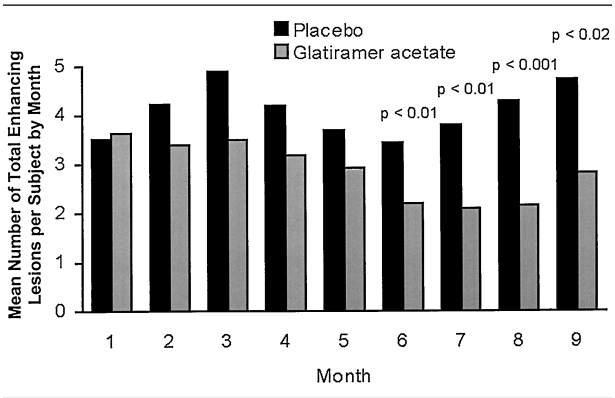


Fig 2. Mean number of total enhancing lesions per subject observed at each month on study using the last observation carried forward approach. Repeated measures analysis favored a treatment effect for glatiramer acetate (p = 0.003). Statistically significant differences first emerged after 5 months of continued therapy from randomization.



marized in Table 2. The percentage of patients with scans free from enhancing lesions at baseline were GA 17.6% and placebo 18.3%. Only six patients in the placebo group and three in the GA treated group were inactive over the entire study. However, on monthly comparison, a positive effect of GA emerged during the third trimester (12.5% versus 27.7%; odds ratio [OR] 3.86; CI 1.76 to 8.48; $p < 0.001$). During the entire study, the mean percentage of scans without any enhancing lesions (i.e., "inactive" scans) was 28.7% (SD = 2.8%) in the placebo group and 35.8% (SD = 2.9%) in the GA-treated group ($p = 0.04$). This difference was not significant during the first trimester of the study (placebo: 29.4 ± 3.4%, GA: 26.2 ± 3.5%), but it became evident during the second (placebo: 29.9 ± 3.6%, GA: 39.8 ± 3.8%, $p = 0.03$) and the third (placebo: 26.0 ± 3.8%, GA: 43.8 ± 3.9%, $p = 0.0002$) trimesters. Repeated measures analysis on the cumulative monthly change from baseline in enhancing lesion volume showed a significant difference that favored active treatment ($p < 0.01$). Figure 3 shows the cumulative enhancing lesion volume on a monthly basis for the entire study duration. The two curves first diverge at month 4 and the separation increased in the following months. The mean total number of new T2 lesions was 13.5 in the placebo group and 9.4 in the GA group, a 30% difference ($p < 0.003$). Differences in the accumulation of new T2 lesions over time in the two groups paralleled those observed for enhancing lesions. By the second trimester, the rate of accumulation of new T2 lesions in the GA-treated group first slowed and then continued to diverge from the placebo group, becoming significant after month 6 (Fig 4). The median percentage change in T2 lesion volume from baseline to the end of the trial was 20.6% in the placebo group and 12.3% in the GA group (a 40% reduction; $p = 0.0011$). Intergroup divergence in the accumulation of T2 lesion volume was again evident during the second trimester and became significant in the third. The median change (baseline to termination), in T1-weighted hypointense lesion volume was

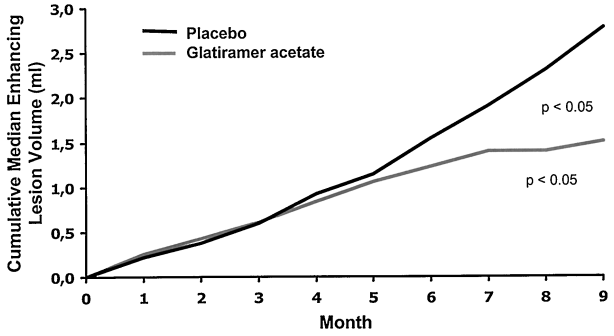


Fig 3. Cumulative median enhancing lesion volume from randomization, displayed in milliliters. Statistically significant differences emerged during the third trimester.

266 μl for the GA arm and 425 μl for the placebo group. This 37% reduction in favor of active treatment was not significant.

Clinical Outcomes

The observed mean relapse rate was 33% lower in the GA group (0.51 relapses/subject) than in the placebo arm (0.76 relapses/subject). This difference was statistically significant ($p = 0.012$). The corresponding annualized relapse rates were 0.81 and 1.21. The numbers of relapses were similar in the two arms over the first two trimesters. In contrast, during the third trimester only five relapses occurred in the GA group, whereas 26 relapses occurred in the placebo group. The proportion of relapse-free patients was slightly increased in the GA group (55.5% versus 49.2%, OR 1.47, CI 0.84 to 2.56; $p = 0.175$). However, the percentage of patients with two or more relapses dropped from 15.8% in the placebo group to 6.7% in the GA group. A significant correlation between cumulative number of enhancing lesions and total number of relapses over the study period was observed in both placebo (Spearman rank correlation coefficient [SRCC] = 0.35; $p = 0.0001$) and in GA-treated patients (SRCC = 0.24, $p = 0.01$). Steroid courses were ad-

Table 2. Secondary MRI End Points

	Placebo		Glatiramer Acetate		
	Mean ± SE	Median	Mean ± SE	Median	p
Total no. new enhancing lesions	26.0 ± 3.1	13.5	17.4 ± 2.2	9.0	<0.003[a]
Change from baseline to month 9 in enhancing lesion volume (μl)	−105.1 ± 177.4	−52.5	−245.3 ± 70.8	−169	0.01
Total no. new T2 lesions	13.7 ± 1.1	8.0	9.4 ± 1.1	5.0	<0.003[a]
Change from baseline to month 9 in T2 lesion volume (ml)	4.7 ± 0.9	3.0	3.0 ± 0.4	1.7	0.006
Change from baseline to month 9 in hypointense T1 lesion volume (ml)	1.3 ± 0.2	0.4	0.8 ± 0.2	0.3	0.14

Values presented are all unadjusted means.

[a]p values refer to comparisons based on adjusted means.

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