Magnetic resonance imaging changes with recombinant human interferon- β -1a: a short term study in relapsing-remitting multiple sclerosis

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Abstract

Objective—To evaluate whether recombinant human interferon- β -1a significantly affects disease activity as measured by a reduction in the number and volume of Gd enhancing lesions on monthly MRI. The study also evaluated the effect on six-monthly T2 weighted abnormality and relapse frequency.

Methods—After a baseline scan and a six month pretreatment period, 68 patients were randomly assigned to receive either 3 MIU or 9 MIU of interferon- β -1a by subcutaneous injection three times a week for six months. All patients were examined by Gd enhanced MRI every month in both pretreatment and treatment periods. The evaluation of Gd enhancing lesions was performed blind at the end of the study.

Results—The mean number of Gd enhancing lesions was higher during the pretreatment period than during treatment. This difference was statistically significant for the two different dose subgroups (3.5 v 1.8, $P < 0.001$ for the 3 MIU group and 2.4 v 0.9, $P < 0.001$ for the 9 MIU group, corresponding to a reduction of 49% and 64% respectively). The mean volume of Gd enhancing lesions also significantly decreased by 61% (3 MIU group) and 73% (9 MIU group). These reductions were evident only after the first month of treatment. The six-monthly rate of new lesions as seen in T2 weighted images showed a similar trend of reduction with treatment (65% and 70% respectively). Lesion volume on T2 scans significantly increased during the pretreatment period whereas it remained almost stable during the treatment period in both groups. Clinical relapse rate was significantly reduced by treatment (53% for the 3 MIU group, $P < 0.001$; 69% for the 9 MIU group, $P < 0.001$).

Conclusion—Interferon- β -1a seemed effective in reducing disease activity in relapsing-remitting multiple sclerosis at both the doses used.

(*J Neurol Neurosurg Psychiatry* 1996;61:251-258)

Keywords: relapsing-remitting multiple sclerosis; recombinant interferon- β ; magnetic resonance imaging; gadolinium enhancement

approach to the objective quantification of the pathology of multiple sclerosis. It is now established that serial brain MRI at monthly intervals shows disease activity in the absence of clinical change and consequently should be helpful in monitoring therapeutic response in small patient populations studied over a short period.¹⁻⁹ In addition, the use of gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA) with T1 weighted imaging greatly increases the sensitivity of monthly brain MRI.¹⁰ Gd enhancement occurs not only in most new lesions appearing during serial monthly scans of patients with relapsing-remitting multiple sclerosis, but may also be seen in older lesions which appear unmodified on T2 weighted MRI.³⁻⁴ Furthermore, Gd enhancement improves the clarity of small lesions, particularly in the cortex, and is well tolerated on serial administration.¹¹

Two large, multicentred, prospective, double blind placebo controlled clinical studies have recently documented the clinical efficacy of systemic treatment with two forms of recombinant interferon- β in patients with relapsing-remitting multiple sclerosis.^{12,13} Interferon- β -1a, which is expressed by a mammalian cell line, is glycosylated and is otherwise identical to the native molecule. Interferon- β -1b is expressed by bacterial cells, is not glycosylated, lacks the N-terminal methionine found in the native molecule, and has a serine substitution for cysteine at position 17.¹⁴

Magnetic resonance imaging has been used as a surrogate marker of activity in both trials and the results supported the clinical findings. Interferon- β -1b significantly decreased disease activity as measured by brain lesion load and by the appearance of new lesions on six-weekly unenhanced MRI examinations.^{12,15} Interferon- β -1a significantly reduced the number of enhancing lesions in annual Gd enhanced MRI studies.¹³ There is also some recent evidence that interferon- β -1b reduces the mean monthly frequency of enhancing lesions as measured in a group of 14 patients with relapsing-remitting multiple sclerosis.¹⁶

The present trial was designed to assess whether human interferon- β -1a at the doses of both 3 MIU and 9 MIU subcutaneously three times a week may have a significant effect on disease activity as measured by a reduction in the mean number and volume of Gd enhancing lesions seen on monthly MRI. The study also evaluated the effect on six-monthly T2 weighted abnormality and relapse frequency.

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Received 5 October 1995
and in final revised form
22 April 1996
Accepted 25 April 1996

The advent of MRI has provided a powerful

Materials and methods

The trial was conducted at the University "La Sapienza" and at the S Camillo Hospital of Rome. The study was approved by the ethics committee of the two centres and written informed consent was obtained from all patients.

STUDY DESIGN

All eligible patients had been under clinical observation for at least two years before the study to assess the diagnosis and record the clinical relapses. After a baseline scan (not used in patient selection) and a six month pre-treatment period, the patients were randomly assigned to receive either 3 or 9 MIU of interferon- β -1a by subcutaneous injection three times a week for six months. The main objective of the study was not to compare the two doses but to assess treatment efficacy of each of the two doses in comparison with the pre-treatment period. All patients were examined by Gd enhanced MRI every month for the entire duration of the study.

INCLUSION AND EXCLUSION CRITERIA

Patients fulfilling the following criteria were included into the study: age between 15 and 45 years, clinically definite or laboratory supported definite relapsing-remitting multiple sclerosis, duration of the disease less than 10 years, disability from 1 to 5 on the Kurtzke expanded disability status scale (EDSS),¹⁷ at least two relapses in the previous two years which were documented in source records of our centres.

Patients were excluded if they were in clinical relapse or under treatment with corticosteroids during the past three months or had immunosuppressive treatment during the past 12 months. Other neurological or psychiatric diseases, pregnancy, and not practicing contraception were also considered as exclusion criteria.

STUDY MEDICATION

The drug used, interferon- β -1a (Rebif, Ares Serono), is produced in Chinese hamster ovary cells and it is identical to the native molecule (fibroblast derived human interferon- β) at both the biochemical and the molecular level (data on file at Ares Serono). In addition, the same pharmacodynamic pattern has been found when the two drugs were compared in a phase I study in healthy volunteers.¹⁸ Interferon- β -1a was supplied in glass vials containing 3 MIU as lyophilised powder to be dissolved in 1 ml of saline solution. The same quantity of solvent (1 ml) was utilised for the 9 MIU dose. Patients were treated on an outpatient basis. Before starting treatment patients were trained to self administer the drug.

PATIENT EVALUATION AND CONCOMITANT TREATMENT

Neurological examination was performed and disability scored on the EDSS at entry, at the end of the pretreatment period, and after the treatment period. Monitoring and recording of relapses, concomitant treatment, or other

medical events were documented throughout the study.

A relapse was defined as the appearance of a new symptom or worsening of an old symptom attributable to multiple sclerosis, accompanied by a documented new neurological abnormality lasting more than 48 hours and preceded by stability or improvement for at least 30 days. In the case of relapse short courses of intravenous methylprednisolone (IVMP) (1 g daily for six days) were allowed.

SAFETY END POINTS, DOSAGE ADJUSTMENT, AND WITHDRAWAL CRITERIA

The safety of the treatment was assessed on the basis of adverse events reported spontaneously by the patient. Furthermore, patients were queried by the treating neurologist with unstandardised non-leading questions every month. The main laboratory variables were monitored every three months.

All patients who received at least one dose of study drug were eligible for the safety analysis. The number and severity of adverse events, local tolerance at injection site, and the occurrence of laboratory abnormalities were documented. Toxicity was evaluated on the basis of the World Health Organisation (WHO) criteria and if significant toxicity occurred, study medication was stopped and resumed at 50% of the initial dose.

ASSESSMENT BY MRI

Brain MRI examinations were performed monthly with a superconductive 0.5 T magnet (Toshiba 50 S) for a total of 13 scans per patient. All scans were taken with T2 spin echo sequences (TR 2500, TE 30/90) on an axial plane. The enhanced study was performed using T1 weighted sequences (TR 400, TE 18) on an axial plane, 15 minutes after injection of Gd-DTPA (0.1 ml/kg). Slices of 5 mm thickness with a 1 mm gap between sections were obtained for all the sequences, using a 25 cm field of view and a 256 $\times$ 160 matrix with two excitations. Reproducible head positioning from month to month was assured by performing a midline sagittal scout slice at the beginning of each study. Therefore, we were able to orient axial sections on the same horizontal plane along a line passing through the base of the frontal lobe and the caudal portion of the quadrigeminal plate. All MRI was carried out in the same neuroradiological unit according to a standardised procedure by a well trained technician.

EVALUATION OF MRI

To avoid potential bias in the treatment effect because treatment always follows the non-treatment period, the evaluation of Gd enhancing lesions on T1 weighted images was done in blind conditions. Blindness was obtained by replacing the dates on scans with codes. This procedure was followed both for the pretreatment and the treatment periods. For each patient, all the images were read at the end of the study in random order, according to a list of random numbers generated by statistical software.¹⁹

Gd enhancing lesions on T1 weighted images were identified by two neuroradiologists independently who traced the lesion outlines on a computer console using dedicated software. The presence of Gd enhancing lesions was identified directly from computer tapes by consensus of the two neuroradiologists. In cases of disagreement, a third senior neuroradiologist also reviewed the tapes, and a final consensus was reached. The Gd enhancing lesions were then displayed directly from computer tapes on a high resolution graphics monitor, and the regions of interest of the previously marked lesions were considered for the volumetric evaluation. Semiautomated detection of the lesion volume was achieved using two thresholds; the first set at a level which best separated brain from surrounding tissues and the second at the optimum level to detect lesions.²⁰ Once the brain was isolated from surrounding tissue, the selected lesion intensity threshold was applied and the remaining regions with intensities above this were labelled as lesions. Regions smaller than four pixels were excluded as these were likely to be due to methodological problems. The computed lesion load was always reviewed manually to correct any errors in lesion detection. As both segmentation techniques operate on individual slices, their results were stated and analysed as areas rather than volumes. The areas stated were multiplied by the slice thickness for a straightforward estimate of volume.

The number of enhancing scans (a scan with at least one enhancing lesion) was also calculated.

The number and volume of Gd enhancing lesions on monthly T1 weighted images during the pretreatment period (from the second to the seventh scan) and those obtained during the treatment phase (from the eighth to the 13th scan) were compared. The first scan (baseline) was not used for this analysis.

After the evaluation of Gd enhancing lesions, the code was broken and T2 weighted images were ordered and paired for the analysis. The number of new lesions and the change in total lesion volume from baseline to the seventh scan (pretreatment period) and from the seventh to the 13th scan (treatment period) were compared.

EFFICACY END POINTS

Treatment efficacy was assessed by comparing the following variables in the pretreatment and treatment periods: (1) primary end points: the mean number and volume of Gd enhancing

lesions on monthly T1 weighted images, (2) secondary end points: the mean number of new lesions and the change in lesion volume on six-monthly T2 weighted images, the mean number of Gd enhancing scans, the mean frequency of clinical relapses, the number of relapse free patients, and the number of steroid pulses.

STATISTICAL METHODS

Calculation of the sample size was based on data published by McFarland *et al.*⁶ According to an α error of 0.05 and a β error of 0.20 (test sensitivity and power = 95% and 80%, respectively), and assuming a 50% decrease in the number of enhancing lesions for each arm necessary to define the efficacy of the treatment, the number of patients required to achieve statistical significance was 35 patients per arm. This sample size takes account of possible dropouts (15%) and allows a comparison of the treatment with pretreatment periods for each of the two doses. However, the number of patients studied in each dose group precluded any direct comparison between the two doses.

The comparison between the pretreatment and treatment periods for both MRI and clinical (number of relapses) variables was by Student's paired *t* test. The Wilcoxon signed rank test was used for the individual dose evaluations because of the smaller patient number. The α error was set to 0.05 two tailed. The McNemar test was also used to assess the changes in the relapse status of the patients during the pretreatment and treatment periods. Relations between clinical relapses and MRI findings were investigated by means of Pearson's correlation coefficients.

Results

STUDY POPULATION

Seventy two patients with relapsing-remitting multiple sclerosis were recruited from June 1993 to January 1994. Five patients dropped out: four during the pretreatment period and one after 40 days of treatment. The patients who dropped out during the pretreatment period were not randomised to treatment phase and were excluded from the analysis of efficacy and safety as they did not receive the drug. Two of these spontaneously withdrew consent because they were unable to comply with the protocol procedures. The other two dropped out because of a breast nodule in one and breast cancer in one, which required further investigation.

Table 1 Baseline clinical and MRI characteristics of patients

	3 MIU group (n = 35)	9 MIU group (n = 33)	Total (n = 68)
Sex (F/M)	27/8	20/13	47/21
Age (y) (mean (SD))	30.5 (8.3)	30.5 (6.2)	30.5 (7.3)
Disease duration (y) (mean (SD))	4.8 (2.4)	5.3 (3.1)	5.0 (2.9)
EDSS (mean (SD))	2.4 (1.0)	1.9 (0.8)	2.2 (0.9)
Relapses in prior 2 years (mean (SD))	3.2 (1.4)	3.1 (1.3)	3.1 (1.3)
Last relapse before entry (months) (mean (SD))	4.9 (2.9)	5.4 (3.6)	5.1 (3.2)
No of Gd enhancing lesions (baseline scan) (mean (SD))	2.5 (4.6)	1.1 (1.7)	1.8 (3.6)
Volume (cm ³) of Gd enhancing lesions (baseline scan) (mean (SD))	0.32 (0.51)	0.14 (0.21)	0.23 (0.4)

MIU = Million international units. There were no significant differences between the two dose groups (Student's unpaired *t* test).

Table 2 Number and volume of Gd enhancing lesions/month/patient

	3 MIU group (n = 35)	9 MIU group (n = 33)	Total (n = 68)
<i>No of Gd enhancing lesions/month/patient</i>			
Pretreatment period (1–6 months; 407 scans):			
Median	1.7	0.7	1.5
Mean (SD)	3.5 (5.0)	2.4 (3.5)	3.0 (4.3)
Treatment period (7–12 months; 399 scans):			
Median	0.8	0.2	0.3
Mean (SD)	1.8 (2.6)***	0.9 (1.7)***	1.3 (2.2)***
<i>Volume of Gd enhancing lesions/month/patient (cm³)</i>			
Pretreatment period (1–6 months):			
Median	0.25	0.10	0.19
Mean (SD)	0.56 (0.82)	0.38 (0.52)	0.47 (0.69)
Treatment period (7–12 months):			
Median	0.09	0.01	0.03
Mean (SD)	0.22 (0.36)***	0.10 (0.18)***	0.16 (0.29)***

***P < 0.001; for within dose evaluation Wilcoxon signed rank test; for total evaluation Student's paired *t* test.

An intention to treat analysis was then performed on the remaining 68 patients. Table 1 shows the baseline demographic, clinical, and MRI characteristics of the 68 patients randomised to the two treatment arms. Although, there were no significant differences between the two groups, the 3 MIU group was slightly more disabled and had a higher number and volume of Gd enhancing lesions at baseline scan, which approached significance ($P = 0.10$ and 0.06 respectively).

EFFICACY OF MRI

Table 2 shows the mean number and volume of monthly Gd enhancing lesions during the study. The mean number of Gd enhancing lesions/month/patient was higher during the pretreatment period than during treatment. This difference was significant for the whole

group (3.0 v 1.3 , $P < 0.001$ by Student's paired *t* test, corresponding to a 55% reduction) as well as for the two different dose subgroups (3.5 v 1.8 , $P < 0.001$ for the 3 MIU group and 2.4 v 0.9 , $P < 0.001$ for the 9 MIU group by Wilcoxon's signed rank test, corresponding to a reduction of 49% and 64% respectively). The mean volume of Gd enhancing lesions/month/patient was 0.47 cm³ during the pretreatment period and 0.16 cm³ during treatment ($P < 0.001$) with a reduction of 66%. Both the doses were effective. There was a reduction of 61% for the 3 MIU group (0.56 cm³ v 0.22 cm³, $P < 0.001$) and of 73% for the 9 MIU group (0.38 cm³ v 0.10 cm³, $P < 0.001$).

Figure 1 shows the mean number (1A) and volume (1B) of Gd enhancing lesions/patient for each month throughout the study in the two groups. A progressive decrease in the number and volume of Gd enhancing lesions was evident after the first month of treatment. The pattern of activity was unchanged during the pretreatment period (non-significant slope) but there was a continuous reduction in activity during the treatment period which was significant ($r = 0.125$; $P = 0.012$; fig 2).

Out of the 68 patients examined, seven (10%) did not show Gd enhancing lesions during the study period. An increase in the number of Gd enhancing lesions during the treatment period was seen in eight (12%) patients (four for each dose group), whereas a complete disappearance of enhancement was seen in 12 (18%) patients (five and seven for the 3 MIU and 9 MIU groups respectively). A reduction in the number of Gd enhancing lesions ranging from 0 to 99% was detected in the remaining 41 (60%) patients (24 and 17 for the 3 MIU and 9 MIU groups respectively). Similar values were noted for the volume of Gd enhancing lesions.

Treatment with IVMP was performed on 61 occasions during the study. In view of the possible effect of steroids on Gd enhancement we repeated the statistical analysis on the number and volume of total Gd enhancing lesions, excluding the first scan after each IVMP treatment (61 out of 806 scans; table 3). The results were similar to those obtained in the previous analysis.

The number of enhancing scans was 238/407 (58%) during the pretreatment period and 145/399 (36%) during treatment. The number of enhancing scans was reduced

Figure 1 (A) Number and (B) volume of monthly Gd enhancing lesions/patient (mean (SEM)) during pretreatment and treatment periods for both the 3 MIU group (dashed lines) and the 9 MIU group (solid lines).

Figure 2 Number of monthly Gd enhancing lesions/patient before and during the treatment period for both groups. Linear regression line = solid line; 95% CI for mean predicted values = dashed line. Regression equation (pretreatment): lesions = $2.61 + 0.1 \times \text{months}$, $r = 0.03$, $F = 0.39$, NS. Regression equation (post-treatment): lesions = $2.07 - 0.21 \times \text{months}$, $r = 0.125$, $F = 6.31$, $P = 0.0124$.

during treatment in both dose groups, from 66% (139/210) to 44% (91/205) in the 3 MIU group and from 50% (99/197) to 28% (54/194) in the 9 MIU group. The mean number of enhancing scans significantly decreased with treatment for the whole group as well as for the two different dose subgroups (table 4).

On evaluation of the six-monthly rate of new lesions as seen in T2 weighted images, there was a 67% overall reduction during treatment (4.8 v 1.6 , $P < 0.001$). The difference was significant for both the groups (3 MIU group 5.7 v 2.0 , $P < 0.001$; 9 MIU group 3.9 v 1.2 , $P < 0.001$ corresponding to a reduction of 65% and 70% respectively). Table 5 shows the lesion volume on T2 scans at baseline, 6, and 12 months and its percentage change during pretreatment and treatment periods. This shows a significant mean increase in lesion volume in both groups during the pretreatment phase (3 MIU group: from 14.7 cm^3 to 18.1 cm^3 , $P < 0.001$; 9 MIU group: from 11.7 cm^3 to 12.9 cm^3 , $P = 0.03$) which is more marked in the low dose group. Lesion volume showed minimal changes during the treatment phase in both groups (3 MIU group: from 18.1 cm^3 to 17.6 cm^3 , NS; 9 MIU group: from 12.9 cm^3 to 12.8 cm^3 , NS).

CLINICAL EFFICACY

There were 58 relapses during the pretreatment period and 23 during treatment. The relapse rate per patient was 0.85 (SD 0.95) during the pretreatment period and 0.34 (SD 0.61) during treatment ($P < 0.001$ by Student's paired t test) which corresponds to a 60% reduction. The difference was significant

for both the groups (3 MIU group 0.91 (SD 0.98) v 0.43 (SD 0.70) $P = 0.007$; 9 MIU group 0.79 (SD 0.93) v 0.24 (SD 0.50) $P = 0.003$ corresponding to a reduction of 53% and 69% respectively).

The number of relapse free patients was 32/68 (pretreatment period) and 49/68 (treatment period) ($P = 0.003$ by McNemar's test). However, when the data were separately analysed for each treatment subgroup, the number of relapse free patients significantly increased only in the group receiving the higher dose (17/33 in the pretreatment period v 26/33 in the treatment period; $P = 0.02$).

The total number of methylprednisolone pulses fell from 43 in the pretreatment period (24 in the 3 MIU group and 19 in the 9 MIU group) to 18 in the treatment period (13 in the 3 MIU group and five in the 9 MIU group).

Mean EDSS score went from 2.15 (SD 0.92) at baseline to 2.21 (SD 1.03) at the end of the pretreatment period and 2.22 (SD 1.26) at the end of the treatment period. These changes were not significant.

RELATION BETWEEN MRI AND CLINICAL FINDINGS

The number of relapses was significantly correlated with the number and volume of Gd enhancing lesions in both the pretreatment period (number: $r = 0.35$, $P = 0.003$; volume: $r = 0.37$, $P = 0.002$) and the treatment period (number: $r = 0.53$, $P < 0.001$; $r = 0.46$, $P < 0.001$). Patients with a clinical relapse during the pretreatment period ($n = 36$) showed a similar percentage reduction in the number and volume of Gd enhancing

Table 3 Number and volume of Gd enhancing lesions/month/patient excluding the first scan after corticosteroid treatment

	3 MIU group (n = 35)	9 MIU group (n = 33)	Total (n = 68)
No of Gd enhancing lesions/month/patient			
Pretreatment period (1–6 months; 363 scans):			
Median	1.7	0.5	1.4
Mean (SD)	3.9 (6.3)	2.7 (4.1)	3.3 (5.3)
Treatment period (7–12 months; 382 scans):			
Median	0.8	0.2	0.3
Mean (SD)	2.0 (3.1)***	0.9 (1.9)***	1.5 (2.7)***
Volume of Gd enhancing lesions/month/patient (cm^3)			
Pretreatment period (1–6 months):			
Median	0.23	0.08	0.19
Mean (SD)	0.61 (0.89)	0.43 (0.65)	0.52 (0.78)
Treatment period (7–12 months):			
Median	0.09	0.01	0.03
Mean (SD)	0.25 (0.41)***	0.11 (0.21)***	0.18 (0.33)***

*** $P < 0.001$; for within dose evaluation Wilcoxon signed rank test; for total evaluation Student's paired t test.

Table 4 Number of Gd enhancing scans/patient

	3 MIU group (n = 35)	9 MIU group (n = 33)	Total (n = 68)
No of Gd enhancing scans/patient			
Pretreatment period (1–6 months):			
Median	4	2	4
Mean (SD)	4.0 (1.9)	3.0 (2.3)	3.5 (2.1)
Treatment period (7–12 months):			
Median	2	1	2
Mean (SD)	2.7 (2.0)***	1.8 (1.8)**	2.3 (2.0)***

P = 0.001; *P < 0.001; for within dose evaluation Wilcoxon signed rank test; for total evaluation Student's paired *t* test.

Table 5 Lesion volume on T2 weighted images at baseline, six and 12 months and its percentage change during the study period

	3 MIU group (n = 35)	9 MIU group (n = 33)	Total (n = 68)
Baseline lesion volume (cm ³):			
Median	10.1	7.9	9.0
Mean (SD)	14.7 (13.1)	11.7 (9.6)	13.2 (11.5)
Six month lesion volume (cm ³):			
Median	13.3	8.5	11.1
Mean (SD)	18.1 (15.5)***	12.9 (10.9)*	15.5 (13.6)***
Median % change	31.7	7.6	23.3
Mean % change	23.1	10.3	17.4
12 month lesion volume (cm ³):			
Median	12.7	8.7	10.0
Mean (SD)	17.6 (15.9)†	12.8 (10.7)†	15.2 (13.7)†
Median % change	-4.5	2.4	-9.9
Mean % change	-2.8	-0.8	-1.9

*P = 0.03; ***P < 0.001; †NS; for within dose evaluation Wilcoxon signed rank test; for total evaluation Student's paired *t* test.

lesions with treatment (51% and of 64% respectively) to that seen in patients (n = 32) without clinical relapse during the same period (57% and 64% respectively).

SAFETY

One patient dropped out after 40 days of treatment (3 MIU group) due to the development of cognitive disturbances, mainly in abstract reasoning and visuospatial abilities, which required admission to the hospital. Brain MRI disclosed two new large confluent lesions in the periventricular parieto-occipital white matter bilaterally. As the presence of disease other than multiple sclerosis was excluded on the basis of several investigations, the patient was successfully treated with IVMP, but refused to continue the study.

Treatment was temporarily discontinued in two patients. The first in the 3 MIU group and the second in the 9 MIU group. Discontinuation lasted 10 and 12 days respectively and was due to mild dyspnoea in the first case and severe bronchopneumonia in the second. In one patient the dosage was reduced from 9 MIU to 3 MIU after five months of treatment because of a persistent asthenia.

The most frequent adverse events were: injection site reactions, which occurred in 40% of the 3 MIU group and 61% of the 9 MIU group and a flu-like syndrome which was reported in 31% of the 3 MIU group and 42% of the 9 MIU group. Other relatively frequent adverse events equally distributed across treatment groups were: headache (26%), fever (21%), asthenia (15%), pain (13%), and insomnia (10%). Additional adverse events occurring less often were: depression (6%), irritability (6%), sleepiness (6%), upper respiratory tract infections (4%), nausea (4%), cystitis (4%), and menstrual disorders (4%).

Laboratory abnormalities associated with

interferon- β -1a were mild and transient. Leucopenia was detected in six patients (9%) during treatment (three in the 3 MIU group and three in the 9 MIU group). Two of these had mild leucopenia before starting the treatment. Seven (10%) and 14 (21%) patients had increases in aspartate aminotransferase and alanine transferase respectively during the treatment period compared with three (4%) and five (7%) during the pretreatment period.

Discussion

The two approaches most commonly employed in therapeutic trials are: (a) the parallel group design with one candidate drug and one placebo arm, and (b) the two period crossover design in which each patient receives both the candidate drug and placebo. The power of the two period crossover design compares favourably with that of the parallel groups design. This can be easily explained by the fact that variability between patients is larger than variability within patients.^{6,9} The major limitation of a crossover design to test the efficacy of interferon is that it is not known whether there is a carry over effect when the treatment is discontinued. In such cases a pretest-post-test design such as the one we adopted, in which a pretreatment period is followed by a treatment period would be preferable.^{4,6,16} Limitations of a pretest-post-test design are the potential bias in the treatment effect because treatment always follows the non-treatment arm and the lack of a blinded clinical evaluation.⁶ Hence, we used an MRI variable as the primary end point to evaluate the therapeutic effect of interferon- β -1a, MRI data being more objective than clinical variables and lending themselves to blind evaluation.

Counting the number of enhancing lesions in T1 and semiautomated determination of lesion enhancing volume with an image processing program²⁰ were used to evaluate treatment efficacy. Most enhancing lesions were easily demarcated using software tools so that lesion volume could be reliably determined. By contrast with the number and volume of new enhancing lesions as previously suggested⁴ we considered the total number and volume of enhancing lesions as the most appropriate measure of this study. This decision was made for three main reasons: (1) the evaluation could be blind, as the sequential reading of images was not necessary; (2) their reduction with treatment indicates not only a decrease in the development of new lesions but also a shorter duration of the inflammatory phase; (3) the power of MRI to detect therapeutic effects is increased because their number is greater than the number of new enhancing lesions due to the persistence of some lesions on monthly examination.

Treatment with interferon- β -1a induced a mean reduction of 49% (3 MIU group) and 64% (9 MIU group) in the number of enhancing lesions over a six month period.

The mean volume of Gd enhancing lesions significantly decreased by 61% (3 MIU group)

and 73% (9 MIU group). These values were slightly lower than those recently reported by Stone *et al* with the same study design as ours and 8 MIU interferon- β -1b on alternate days.¹⁶ In their study, the number of total enhancing lesions was reduced by 84% during the treatment period. Different criteria in patient selection, number and clinical characteristics of the patients, as well as in the image evaluation (blind *v* unblind evaluation), may explain the discrepancy between the two studies.

Our results indicate that the number of enhancing lesions are often parallel to lesion volume; however, independent variation in these two variables has been observed reflecting either a number of very small lesions or a few large enhancing lesions. There was also some slight dissociation after treatment. Interferon- β -1a induced a greater decrease in the enhancing volume than in the number of enhancing lesions. Smith *et al*⁸ suggested that different mechanisms influence the number and the area of enhancing lesions and that these two variables may provide independent effects of clinical worsening.

Beneficial effects of interferon- β -1a on MRI were seen only after the first month of treatment (see fig 1A and 1B). There is some suggestion that during the first three months of treatment with interferon- β -1a there may be activation of the disease process as measured by the number of "active" lesions.²¹ By comparing the number of enhancing lesions during the first three months of treatment with interferon- β -1a with those during the three months before treatment, we found an increase in lesions in 8/68 (12%) of the patients. All these patients had a large reduction of enhancing lesions in the next three months of treatment.

Previous studies²²⁻²⁴ showed that treatment with IVMP reduced enhancement on the MRI. Despite sustained clinical improvement, many lesions re-enhance within a few days of stopping the steroids and new lesions often appeared within one month.²⁴ Therefore, to avoid the transient effect of steroids, we repeated a statistical analysis excluding the first scan after steroid treatment (table 3). The reduction in the number and volume of enhancing lesions by interferon- β -1a was still almost identical to that of all scans. These findings are consistent with previous ones indicating that the total number of enhancing lesions and the enhancing lesion area are poorly affected by steroid treatment.⁸

A significant effect of interferon- β -1a was seen also with the secondary no blind MRI end points. We found a decrease in new lesions on six-monthly T2 weighted images in 65% of the patients treated with 3 MIU and in 70% of the patients treated with 9 MIU. These values were only slightly higher than those reported in the interferon- β multiple sclerosis study, in which 52 patients were studied using serial MRI every six weeks.¹⁵ Mean rate of new lesions per year on T2 weighted images were reduced by 62% in the group treated with interferon- β -1b at a dose of 8 MIU on alternate days when compared with placebo. On

the other hand, Stone *et al*¹⁶ reported that the frequency of new lesions on T2 weighted scans was 90% lower during the treatment period than the pretreatment period. Despite these differences, which are likely to be due to trial variability, and according with the study of Stone *et al*,¹⁶ we found that interferon- β -1a had a similar effect on the reduction in enhancing lesions or development of new lesions on T2 weighted scans (64% and 70% respectively in the 9 MIU group). This is not surprising as longitudinal MRI studies have previously shown that almost all enhancing lesions are followed by permanent T2 abnormalities.⁴ Furthermore, we were able to show a significant increase in lesion volume on T2 scans during the pretreatment period (15% over six months) and no remarkable changes were seen during the treatment period in either group (table 5), despite the short duration of the trial. Taken together these data may be relevant for the design of future trials using MRI as an outcome measure. The assessment of the amount of diseased tissue, as measured by the number of lesions or volume of increased signal intensity on T2 weighted scans every six months, seems to be a cost and time saving outcome measure. To be useful, however, correct estimates of sample size, patient selection, and accuracy of lesion load measurement will be particularly important.

In this study, interferon- β -1a significantly reduced clinical disease activity. The relapse rate was decreased by 60% (53% for the 3 MIU group and 69% for the 9 MIU group). Similarly, the patients were more likely to be free of exacerbation during the treatment period (72%) than during the period of observation (47%), although this difference did not reach significance in the group receiving the lower dosage. The use of concomitant steroid treatment was also reduced by 58% in the treatment period in comparison with the pretreatment period.

The trial with interferon- β -1a¹³ reported a reduction in the annual relapse rate from 1.2 to 0.61/year (49%) in patients receiving 6 MIU once a week, compared with a reduction from 1.2 to 0.9/year (25%) in the placebo group (a 32% difference between the two groups). In the interferon- β -1b trial,¹² the relapse rate after two years was 34% lower in patients receiving 8 MIU than in those receiving placebo. However, there was a 51% reduction if the relapse rate of the 8 MIU group was compared with that in the same patients during the two years before the study. Therefore, the difference between our clinical findings and those reported in the two large multicentre, double blind, placebo controlled trials is not great. The use of patients as their own controls and the lack of placebo effect may explain the high reduction of relapse rate seen in our trial.

The significant association between the number of enhancing lesions and relapse rate in this, as in previous studies,^{7,8,25,26} indicates that enhancing lesions are a clinically important component of the disease process and offer an objective outcome measure for assess-

ing response to treatment. The advantage of MRI is that it discloses pathological changes that are 5–10 times more frequent than clinical relapses in patients with relapsing-remitting multiple sclerosis.^{4,7,8} We found a total of 58 clinical relapses against a total of 238 enhancing scans (a scan with at least one enhancing lesion) during the pretreatment period indicating that MRI activity is about four times more frequent than the clinical activity. During the same period 90% (61/68) of the patients showed signs of MRI activity (at least one enhancing scan), whereas only 53% (36/68) experienced a clinical relapse. Out of seven patients without MRI activity, only one had clinical exacerbation related to spinal cord involvement. By contrast, out of 32 exacerbation free patients, 26 showed MRI activity. These data are in agreement with previous findings^{3,4,25} indicating that about 90% of the patients are thought to be active on clinical grounds; MRI changes support this opinion.

The drug was well tolerated by most patients and was easily given to outpatients. No patient required permanent discontinuation of the treatment because of adverse events and only one required dosage reduction from 9 MIU to 3 MIU. Most adverse events were mild and transient. Mild hepatotoxicity with an asymptomatic rise of transaminases always lower than 2.5 times the upper limit of normal was noted in 31% of patients in the treatment period compared with 12% during the pretreatment period.

Although the number of patients studied in each dose group precluded any direct comparison of the two doses, both were well tolerated and significantly effective in reducing MRI variables. This reduction was slightly more evident in the high dose group (49% *v* 64% for number of enhancing lesions; 61% *v* 73% for enhancing volume). On the other hand, patients given the lower dose experienced a slightly lower incidence of some adverse events (40% *v* 61% for injection site reactions; 31% *v* 42% for flu-like symptoms).

In conclusion, this study clearly shows that interferon- β -1a is effective in reducing MRI activity in the short term. This was accompanied by a reduction in the clinical exacerbation rate; however, the study was not designed to assess any possible effect on the progression of disability. On the other hand, there are still no definite clinical data to support the notion that the reduction in MRI activity translates into a reduction in measurable progression of sustained disability. Specific answers to this issue will be hopefully provided by the currently on going international large phase III clinical studies on both relapsing-remitting and progressive multiple sclerosis.

We thank the patients for their cooperation; Dr R D'Antona, Dr M G Grasso, Dr M Salvetti, Dr G Ristori, Dr M Frontoni, Dr A Francia, Dr S Bernardi, Dr M Spadaro, Dr M R Fele, and Professor C Scoppetta for permission to study their patients; Dr A Paolillo, Dr M D'Erme, V Tamburrino, V Bonetti, and M Tarquini for their technical MRI skills throughout the study; M L Brigiari and A Pellicciotti (Scuola Agnelli) for clinical nursing; Professor G Comi, Dr M Filippi, Professor M Prencipe, Professor G L Lenzi for their helpful suggestions; and Dr A J Thompson and Professor H F McFarland for reviewing this manuscript. The Dispimage package display pro-

gram used for the measurement of lesion volume was written by D Plummer, D A G Wicks, P S Tofts, G J Barker, and M A Horsfield, Department of Medical Physics, University College, and MRI Research Group, Institute of Neurology, Queen Square, London. The study has been supported by Ares Sero. We thank Dr R Camerini, Dr L Palmisano, Dr C Pellas, Dr W Groppi, and Dr P Carminati of Ares Sero for their assistance and continuous effort.

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