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T_1 hypointense lesions in secondary progressive multiple sclerosis: effect of interferon beta-1b treatment

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Summary

Recently, the clinical efficacy of interferon β-1b (IFNβ-1b) was demonstrated for secondary progressive (SP) multiple sclerosis in a European multicentre study. We evaluated the effect of IFNβ-1b treatment on the rate of development of hypointense T_1 MRI lesions, a putative marker of axonal damage. Unenhanced T_1-weighted images were obtained in a subgroup of 95 multiple sclerosis patients from five centres at 6-month intervals; this subgroup was similar to the total study population for all demographic, clinical and MRI parameters. An experienced observer blinded to the clinical data and treatment allocation measured volumes. The median baseline lesion load for hypointense T_1 lesions was 5.1 cm^3 for placebo-treated and 4.9 cm^3 for IFNβ-1b-treated patients ($P = 0.56$). Placebo-treated patients showed an increase in T_1 lesion load by a median of 14% per year ($P = 0.0002$ compared with baseline); this was reduced to 7.7% per year in the IFNβ-1b-treated patients ($P = 0.003$ versus placebo). In the IFNβ-1b arm there was a statistically significant correlation between absolute change in Expanded Disability Status Scale scores and T_1 lesion load by month 36 ($r = 0.38$, $P = 0.0015$). In patients with SP multiple sclerosis, IFNβ-1b treatment reduces the development of hypointense T_1 lesions, suggesting that reduced axonal damage in lesions may play a part in the beneficial effect that is observed clinically.

Keywords: multiple sclerosis; interferon beta; MRI; black holes; magnetization transfer

Abbreviations: EDSS = Expanded Disability Status Scale; IFNβ-1b = interferon β-1b; RR = relapsing–remitting; SP = secondary progressive

Introduction

Several types of interferon β have been shown to be partially effective in the treatment of relapsing–remitting (RR) multiple sclerosis (Paty and Li, 1993; Multiple Sclerosis Collaborative Research Group, 1996; Li and Paty, 1999). Recently, efficacy of interferon β-1b (IFNβ-1b) was also demonstrated for secondary progressive (SP) multiple sclerosis in a European multicentre study (European Study Group on interferon beta-1b in secondary progressive multiple sclerosis, 1998), which showed delayed development of sustained progression, a lower relapse rate and reduced MRI evidence of disease. Frequent MRI showed a continuously reduced rate of development of active lesions, and the T_2 lesion load measured in annual scans of all patients was reduced compared with baseline (Miller *et al*., 1999), a result quite

similar to that found for RR patients (Paty and Li, 1993; Li and Paty, 1999).

The mechanisms that lead to relapse and MRI lesion activity may be similar in RR and SP patients, and the congruent results of interferon treatment in the two subtypes may therefore not come as a surprise. With regard to the development of disability, RR patients predominantly suffer from incomplete recovery from relapses, while SP patients, by definition, also suffer from chronic progression independently of relapses. In SP patients, the development of disability is likely to result from more pronounced progressive axonal loss, either in lesions or in the form of widespread non-focal disease.

To gain further insight into the biological mechanisms underlying the slowing of progression in SP patients on IFNβ-1b therapy, we evaluated the development of hypointense MRI lesions under treatment. In comparison with RR multiple sclerosis, hypointense T_1 lesions are more prevalent in SP patients and correlate better with disability than hyperintense T_2 lesions (Truyen *et al*., 1996). Data from post-mortem studies (van Waesberghe *et al*., 1999) and *in vivo* magnetic resonance spectroscopy (van Walderveen *et al*., 1999) indicate that lesion hypointensity is strongly correlated with axonal density, and it is thus well suited to address the rate of development of axonal loss in multiple sclerosis. In this paper we report the effect of IFNβ-1b treatment on the rate of development of hypointense lesions in SP multiple sclerosis.

Material and methods

This study was performed in a subgroup of 95 multiple sclerosis patients participating in a large European, multicentre, randomized, double-blind, placebo-controlled trial (Polman *et al*., 1995; European Study Group on interferon beta-1b in secondary progressive multiple sclerosis, 1998). In brief, patients with SP multiple sclerosis were randomized to receive IFNβ-1b (Betaferon; Schering, Berlin, Germany) 8 MIU, subcutaneously on alternate days, or placebo injections. The study was approved by the Ethical Committees of the participating centres, and all patients gave informed consent. The full study included 718 patients, all of whom underwent yearly conventional T_2-weighted imaging; in a subset of 125 patients from seven centres, frequent gadolinium-enhanced images were also obtained (Miller *et al*., 1999). In five of those centres (Amsterdam, Milan, Munich, Würzburg and London), additional unenhanced T_1-weighted images were obtained at 6-month intervals to assess the development of hypointense lesions. Clinical disability was measured at 3-month intervals using the Expanded Disability Status Scale (EDSS) score by the assessing physician, who was blinded to treatment-related activities (Polman *et al*., 1995). The T_2-weighted and gadolinium-enhanced MRI results have been reported previously (Miller *et al*., 1999), and we used the existing database to assess whether our subset differed from the full study with regard to T_2 lesion load or active lesions.

MR imaging protocol and image analysis

The data used for this study comprised unenhanced T_1-weighted images obtained before the administration of gadolinium. All five centres operated at 1.5 T. T_1-weighted images were obtained using a spin-echo sequence with a repetition time between 400 and 600 ms and echo time between 5 and 25 ms, using 5 mm slices and 1 mm in-plane resolution. To obtain a contiguous data set of 24 slices, two interleaved series of 12 slices each were acquired. On these T_1-weighted images, hypointense lesions were marked using a mouse-controlled cursor on the electronic data by a single experienced observer. Lesion hypointensity was defined as a signal clearly lower than the surrounding white matter, but not necessarily lower than the grey matter. Identification of lesions was done by checking, for each lesion that was visible on a T_2-weighted image, whether it was hypointense on the corresponding T_1-weighted image. The observer marked all scans of a given patient consecutively in order to reduce observer variability, but without knowledge of clinical data and treatment allocation. The marked lesions were then quantified by another observer on the electronic MRI data using home-developed software based on seeding and local thresholding, and the result was multiplied by the interslice distance to obtain volumetric data; the intra-observer variation for the quantification is 5% (Truyen *et al.*, 1996). These observers were fully blinded to the clinical data and treatment allocation.

Statistical analysis

Data were entered into a database by an independent contract research organization (independent from the sponsor) and checked for consistency. Therefore, even though the analyses were performed after unblinding of the study, the analyses were performed in a strictly blinded fashion. Statistical analyses were performed for both absolute and percentage changes in hypointense lesion load compared with baseline. Analyses include all patients with data available at baseline and at least once during treatment. All data available at any given point in time were evaluated with missing data maintained as missing. In additional analyses, missing data were imputed by linear interpolation; because these yielded the same results, only data without replacement are presented in this report. Furthermore, changes in hypointense lesion load at individual last visits (last scan available) were evaluated.

Comparisons of baseline data between groups were performed using the Wilcoxon rank-sum test and Fisher's exact test. To assess changes in hypointense lesion load from baseline within treatment groups, the paired *t* test was used. Comparisons between groups were performed using non-parametric analysis of covariance with stratification

Table 1 *Baseline demographic and disease characteristics of cohort as a whole* (n = *718) and the subgroup evaluated for* T_1 *hypointense lesions* (n = *85)*

Baseline variable	Placebo		IFNβ-1b	
	Cohort (n = 358)	Subgroup (n = 41)	Cohort (n = 360)	Subgroup (n = 44)
Sex (% females)	64.2	39.0	58.1	51.1
Age (years)				
Mean	40.9	39.5	41.1	40.6
SD	7.9	8.8	7.7	8.8
Disease duration (years)				
Mean	13.4	12.7	12.8	11.3
SD	7.5	6.9	6.6	6.4
Time since evidence of progressive deterioration (years)				
Mean	3.8	4.1	3.8	3.4
SD	3.4	2.7	2.7	2.0
Time since diagnosis of SP multiple sclerosis (years)				
Mean	2.1	2.2	2.2	1.4
SD	2.2	1.9	2.3	1.2
Baseline EDSS				
Mean	5.2	5.2	5.1	5.0
SD	1.1	1.0	1.1	1.1
T_2 lesion volume (cm^3)				
Mean	28.4	28.2	26.6	28.0
SD	22.5	21.2	21.2	24.1
Number of enhancing lesions				
Mean	2.2	2.2	3.0	2.7
SD	3.8	3.7	6.6	5.2

adjustment for centre and covariance adjustment for baseline lesion load. To assess the association between T_1 hypointense lesion load and EDSS, non-parametric (Goodman–Kruskal) correlation coefficients were calculated; because we assumed that changes in data for month 6 may have been confounded by temporary hypointense lesions (and minor fluctuations in EDSS), assessments were correlated from month 12 onwards.

Results

For seven patients no valid T_1 data were available and for three others the baseline value was missing, leaving 85 valid cases for analysis, 41 on placebo, 44 on IFNβ-1b. Table 1 shows that the demographics and baseline descriptive findings of those patients were similar to those of the total study population, indicating that no major selection bias had occurred. Also, none of the baseline descriptive data differed between placebo and verum at the 5% significance level. For T_2 lesion load, there was a median increase of 4.19 cm^3 (17.5%) at month 36 in the placebo group, whereas the IFNβ-1b group showed a median decrease of 0.35 (4.0%) at month 36 ($P < 0.0001$ between groups). At month 36, the median cumulative number of new or enlarged lesions on T_2-weighted images was 7 in the placebo group and 1 in the IFNβ-1b group ($P = 0.038$). Similarly, highly significant differences favouring the IFNβ-1b group were seen in the numbers of enhancing lesions during the monthly scanning blocks for months 1–6 and 19–24 ($P < 0.0005$ between groups for both periods). All these data are consistent with the analysis performed on the full data set (European Study Group on interferon beta-1b in secondary progressive multiple sclerosis, 1998).

The median baseline lesion load for hypointense T_1 lesions was 5.1 cm^3 for the placebo group and 4.9 cm^3 for the IFNβ-1b patients ($P = 0.56$). Both arms showed a linear increase in lesion load across time (Table 2). In the placebo-treated patients, this increase was significant from month 6 onwards; by month 36 they had increased their T_1 lesion load by a median value of 2.43 cm^3, corresponding to a median change from baseline of 41.8% ($P = 0.0002$ compared with baseline), with an average an increase of 14% per annum (Fig. 1). The IFNβ-1b patients also showed a linear increase from baseline, which became statistically significant at month 18 for the first time, and by month 36 the median increase in lesion load was 0.76 cm^3, corresponding to a median change from baseline of 23.2% ($P = 0.006$), with an average increase of 7.7% per annum. The rate of increase in T_1 hypointense lesion load was significantly slower in the IFNβ-1b treated patients than in the placebo-treated group ($P = 0.0003$). This was also true when individual last-scan data were analysed ($P = 0.0001$, adjusted value for treatment effect between groups).

The correlations between absolute change in T_1 lesion load and EDSS are reported in Table 3. For the whole group, there was a positive correlation ($r = 0.28$ at month 36, $P = 0.007$). Within the individual arms, the correlations were weak in the placebo arm, whereas statistically significant correlations were found in the IFNβ-1b arm from month 12

Table 2 *Hypointense lesion load: baseline findings and percentage change from baseline*

	Placebo	IFNβ-1b	*P**
Baseline volume			
n	41	44	
Mean (SD)	8.9 (8.1)	7.8 (8.3)	
Median	5.1	4.9	0.5586‡
Percentage change from baseline volume			
Month 6			
n	39	43	
Mean (SD)	16.6 (40.2)	2.7 (18.6)	
Median	9.0	2.2	
P†	0.0140	0.3457	0.0943
Month 12			
n	39	43	
Mean (SD)	19.5 (29.5)	2.5 (22.9)	
Median	11.1	2.7	
P†	0.0002	0.4718	0.0064
Month 18			
n	34	39	
Mean (SD)	28.8 (34.9)	8.7 (26.4)	
Median	24.2	8.5	
P†	<0.0001	0.0474	0.0108
Month 24			
n	34	41	
Mean (SD)	35.6 (43.0)	12.3 (31.6)	
Median	29.5	9.6	
P†	<0.0168	0.0168	0.0009
Month 30			
n	26	30	
Mean	52.4 (54.1)	8.0 (32.1)	
Median	39.1	13.9	
P†	<0.0001	0.1850	0.0002
Month 36			
n	29	37	
Mean	60.0 (78.0)	17.4 (36.5)	
Median	41.8	23.2	
P†	0.0002	0.0063	0.0003
Last visit			
n	41	44	
Mean (SD)	57.5 (70.8)	14.9 (35.1)	
Median	38.6	19.5	
P†	<0.0001	0.0075	0.0001

*Non-parametric analysis of covariance with stratification adjustment for centre and covariance adjustment for baseline hypointense T_1 lesion load; †*t* test for significance of within group change from baseline; ‡Wilcoxon rank-sum test for comparison between groups.

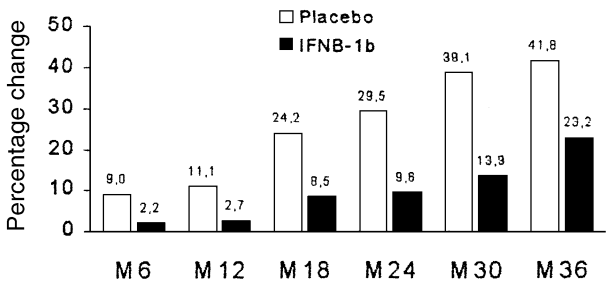

Fig. 1 Median percentage change in hypointense T_1-lesion load from baseline. M = month.

Table 3 *Correlations (*r*) between change in T_1 hypointense lesion load and EDSS*

	n	Placebo	IFNβ-1b	Overall
Month 12	82	0.091	0.317**	0.184*
Month 18	70	−0.10	0.467***	0.203*
Month 24	73	−0.051	0.290*	0.091
Month 30	56	0.072	0.496***	0.287*
Month 36	62	0.269	0.381**	0.284**
Last visit	85	0.170	0.335***	0.214*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

onwards ($r = 0.38$ at month 36, $P = 0.002$). In the same subgroup of patients, no significant correlations were detected between EDSS and T_2 lesion load (not tabulated), with a maximum correlation of 0.16 for the IFNβ-1b arm after 3 years ($P = 0.19$).

Discussion

Traditionally, MRI measures used in treatment trials include the number of active (e.g. gadolinium-enhancing or new T_2) lesions and changes in T_2 lesion load. The behaviour of these MRI measures has been fairly well established, and they provide high statistical power in detecting anti-inflammatory treatment effects (Sormani *et al*., 1999; Molyneux *et al*., 2000*a*). MRI-monitored interferon-beta studies have systematically shown dramatic treatment effects on active lesions and T_2 lesion load, typically with a dose–effect relationship (Paty and Li, 1993; Multiple Sclerosis Collaborative Research Group, 1996; Li and Paty, 1999; Miller *et al*., 1999). On the basis of these and other results, an *ad hoc* committee of the National Multiple Sclerosis Society of the USA decided to advocate the use of MRI measures as a primary outcome measure in exploratory (phase II) trials (Miller *et al*., 1996). However, given the uncertain relationship between changes in MRI and clinical parameters over time, it also recommended that MRI should only be used as a secondary outcome measure in definitive (phase III) trials (Miller *et al*., 1996).

There are many problems in detecting a relationship between MRI measures and clinical status. Apart from problems with clinical scales, the location of lesions and the duration of follow-up, the lack of histopathological specificity beyond the inflammatory stage (identified by gadolinium enhancement) is a major weakness of MRI. On T_2-weighted images, almost any alteration in brain tissue composition will lead to increased signal, including oedema, partial demyelination, gliosis and axonal loss. There is evidence that even remyelinated lesions may return high signal on these T_2-weighted images ('t Hart *et al*., 1998; van Walderveen *et al*., 1998). This could explain the poor correlation with clinical disability, which is likely to be determined mainly by axonal loss. There are several magnetic resonance techniques with improved histopathological specificity, including magnetic resonance spectroscopy, magnetization

transfer and T_1 hypointensity. In this study we evaluated the effect of IFNβ-1b treatment on T_1 hypointensity as a marker of matrix destruction and axonal loss in progressive multiple sclerosis (van Walderveen *et al*., 1998, 1999; van Waesberghe *et al*., 1999).

The subset of patients presented here is comparable with the full data set (European Study Group on interferon beta-1b in secondary progressive multiple sclerosis, 1998), indicating that no significant selection bias occurred and that the treatment arms were well matched. Valid T_1 data were available for the vast majority of patients. The T_1 lesion load at baseline was slightly higher than in an earlier study on the natural history of multiple sclerosis (Truyen *et al*., 1996), in which a median volume of 3.0 cm^3 was reported for SP multiple sclerosis patients, who progressed to 4.15 cm^3 after an average of 3.5 years. The increase of 12% per annum in that natural course study is similar to the increase in lesion load in the placebo arm of the present study (42% over 3 years). The finding of an expected pattern of behaviour in the placebo arm is an essential element in the interpretation of randomized controlled trials (Li and Paty, 1999).

In studies of SP multiple sclerosis, the increase in T_1 hypointense lesion load may seem large with respect to the increase in T_2 lesion load (Truyen *et al.*, 1996); in our study the median increase in T_1 hypointense lesion load was 2.43 cm^3, while the T_2 hyperintense lesion load increased by a median of 4.19 cm^3. This may reflect three phenomena. First, the net change in lesion load is derived from an increase by new lesions and shrinkage by others (Lee *et al.*, 1998), a phenomenon that is much less plausible for T_1 hypointense lesions. Secondly, progressive damage in pre-existing lesions has been demonstrated (Rocca *et al*., 1999), suggesting that ongoing axonal damage may occur in pre-existing lesions, especially in patients with SP multiple sclerosis, which may eventually lead to hypointensity on T_1-weighted SE images. Lastly, the relatively small lesion load at baseline compared with the T_2-hyperintense lesion load may influence the increase in T_1 hypointense lesion load, expressed as a percentage. The latter phenomenon may also explain the findings in patients with RR multiple sclerosis, with a yearly increase of 14.5% per year (Simon *et al.*, 2000), the low lesion load at baseline (median 0.64 cm^3) providing a small denominator.

In the IFNβ-1b-treated arm, the increase in T_1 lesion load was significantly lower than in the placebo arm ($P = 0.0003$), with a 45% reduction in the increase in T_1 hypointense lesion load. This is the first parallel-design controlled study to demonstrate a therapeutic effect on this MRI parameter in SP multiple sclerosis patients. An effect on the development of hypointense lesions has also been noted in a phase II trial of interferon alpha-1a in RR multiple sclerosis, in which patients served as their own controls (Gasperini *et al*., 1999). In this phase II study, a lower percentage of new lesions on T_2-weighted images with persistent hypointensity on T_1-weighted images was suggested (Paolillo *et al*., 1999*a*). A phase III trial with interferon alpha-1a failed to find a significant treatment effect, even though a clear trend was suggested (Simon *et al*., 2000).

Both post-mortem MRI data (van Waesberghe *et al*., 1999) and *in vivo* magnetic resonance spectroscopy data (van Walderveen *et al*., 1999; Brex *et al*., 2000) indicate that the degree of T_1 hypointensity relates to the amount of axonal damage (resulting in widening of extracellular space, with more free, slowly relaxing water). The smaller increase in T_1 hypointense lesion load in the IFNβ-1b group in our SP multiple sclerosis study indicates less progression of axonal loss in lesions. As axonal loss is the most likely correlate of clinical progression, our findings are comparable with the moderate clinical effect of 40% reduction in time to progression (12 months delay after a median time on study of 18 months (European Study Group on interferon beta-1b in secondary progressive multiple sclerosis, 1998). The causality between these two measures is suggested by the finding of positive correlation coefficients in the IFNβ-1b arm; the absence of similar correlations in the placebo arm could be explained by more relapse-related changes in disability. The fact that even in the IFNβ-1b arm the correlations are still modest indicates that other mechanisms leading to disability (e.g. diffuse disease leading to atrophy) may still be at play.

The 45% reduction in development in T_1 hypointense lesions is closer to the clinical effect size than the enormous effect seen on T_2 lesion load; the latter even showed a slight decrease in lesion load in the IFNβ-1b arm after 3 years (Miller *et al*., 1999). The overestimation of the treatment effect on T_2 lesion load relates to an initial decrease in lesion load, which has been a consistent finding in previous interferon studies (Paty and Li, 1993; Li and Paty, 1999) . This probably reflects the resolution of oedematous and inflammatory changes caused by active lesions present at baseline, and the subsequent increase in T_2 lesion load during the trial is too small to offset this initial dip. A similar phenomenon is not detectable for the development of T_1 hypointensity using this 6-monthly analysis, even though monthly studies have noted that a percentage of lesions may become hypointense initially (van Waesberghe *et al*., 1998; Rovira *et al*., 1999). However, the vast majority of the temporary hypointense lesions revert to isointensity after 1 or 2 months, which probably explains why lesion load measurements of unenhanced and gadolinium-enhanced images (where acutely hypointense lesions are masked by their enhancement) show an extremely high correlation (O'Riordan *et al.*, 1998). Therefore, in this study, with a low sampling rate (relative to the duration of enhancement and oedema of new multiple sclerosis lesions), we are confident that the increase in hypointense lesions measured in this study was not due to temporary hypointensity (with marked oedema), but rather to the development of chronic 'black holes' (with axonal loss).

Previous studies in SP multiple sclerosis have shown a relationship between change in the T_1 hypointense load and the rate of atrophy (Paolillo *et al*., 1999*b*). The fact that such

measures are correlated indicates that both are (indirect) measures of axonal damage. On the other hand, the correlations are far from perfect, indicating that severe tissue damage in lesions (i.e. black holes) and overall cerebral tissue loss are not invariably linked. This partial independence may also account for the disparate effect of INFβ-1b on a measure of cerebral volume in the same trial with SP multiple sclerosis patients (Molyneux *et al.*, 2000*b*). In that study, a progressive decrease in cerebral volume was noted in both placebo- and interferon-treated patients, with no clear treatment effect. The partial effect on T_1 hypointense lesions in the absence of a clear effect on cerebral volume loss indicates that, in this group of SP multiple sclerosis patients, axonal loss may progress to a certain extent. Similar uncoupling has been noted with other immunomodulatory drugs that have a clear effect on gadolinium enhancement, but no effect on T_1 hypointense lesion load and atrophy (Coles *et al.* 1999; Filippi *et al.*, 2000).

In conclusion, this is the first phase III trial to show a beneficial effect of IFNβ-1b on the development of T_1 hypointense lesion load, a marker of axonal loss in the lesions of SP multiple sclerosis.

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