Effect of statins on clinical and molecular responses to intramuscular interferon beta-1a

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ABSTRACT

Background: Findings from a small clinical study suggested that statins may counteract the therapeutic effects of interferon beta (IFNβ) in patients with relapsing-remitting multiple sclerosis (RRMS).

Methods: We conducted a post hoc analysis of data from the Safety and Efficacy of Natalizumab in Combination With IFNβ-1a in Patients With Relapsing-Remitting Multiple Sclerosis (SENTINEL) study to determine the effects of statins on efficacy of IFNβ. SENTINEL was a prospective trial of patients with RRMS treated with natalizumab (Tysabri®, Biogen Idec, Inc., Cambridge, MA) plus IM IFNβ-1a (Avonex®, Biogen Idec, Inc.) 30 μg compared with placebo plus IM IFNβ-1a 30 μg. Clinical and MRI outcomes in patients treated with IM IFNβ-1a only (no-statins group, n = 542) were compared with those of patients taking IM IFNβ-1a and statins at doses used to treat hyperlipidemia (statins group, n = 40).

Results: No significant differences were observed between treatment groups in adjusted annualized relapse rate ($p = 0.937$), disability progression ($p = 0.438$), number of gadolinium-enhancing lesions ($p = 0.604$), or number of new or enlarging T2-hyperintense lesions ($p = 0.802$) at 2 years. More patients in the statins group reported fatigue, extremity pain, muscle aches, and increases in hepatic transaminases compared with patients in the no-statins group. Statin treatment had no ex vivo or in vitro effect on induction of IFN-stimulated genes.

Conclusions: Statin therapy does not appear to affect clinical effects of IM interferon beta-1a in patients with relapsing-remitting multiple sclerosis or the primary molecular response to interferon beta treatment. ***Neurology*® 2009;72:1989–1993**

GLOSSARY

ANCOVA = analysis of covariance; **CI** = confidence interval; **EDSS** = Expanded Disability Status Scale; **GAPDH** = glyceraldehyde-3-phosphate dehydrogenase; **Gd+** = gadolinium-enhancing; **IFNβ** = interferon beta; **ISG** = IFN-stimulated gene; **RRMS** = relapsing-remitting multiple sclerosis; **SENTINEL** = Safety and Efficacy of Natalizumab in Combination With IFNβ-1a in Patients With Relapsing-Remitting Multiple Sclerosis.

In addition to interfering with cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) are pleiotropic compounds with anti-inflammatory, immunomodulatory, and antithrombotic effects.[1] Statins are beneficial in experimental autoimmune encephalomyelitis, an animal model of relapsing-remitting multiple sclerosis (RRMS).[2,3] We conducted a post hoc analysis of the Safety and Efficacy of Natalizumab in Combination With IFNβ-1a in Patients With Relapsing-Remitting Multiple Sclerosis (SENTINEL) study of natalizumab[4] (Tysabri®, Biogen Idec, Inc., Cambridge, MA) to explore the effects of statins on the

Supplemental data at www.neurology.org

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Table 1 Demographic characteristics and baseline disease severity

Characteristic	No statins (n = 542)	Statins (n = 40)
Age, y, mean (SD)	38.7 (7.56)	44.0 (7.15)
Statins	0	40*
Atorvastatin	0	26
Simvastatin	0	13
Pravastatin†	0	3
Lovastatin‡	0	2
Fluvastatin§	0	1
Median duration of MS, y (min, max)	8 (1, 34)	8 (1, 31)
EDSS score, mean (SD)	2.5 (1.13)	2.6 (1.13)
Relapses in prior year, mean (SD)	1.5 (0.72)	1.4 (0.71)
Median T2 lesion volume, mL^3 (min, max)	5,130.2 (0, 84,899)	4,394.8 (235, 28,674)
Gd+ lesions, median (min, max)	0 (0, 16)	0 (0, 2)
Gd+ lesions, %	37	18

*The total number of statins exceeds 40 because five patients took more than one statin over the course of the trial.
†Pravachol®, Bristol-Myers Squibb Company, Princeton, NJ.
‡Mevacor®, Merck & Co., Inc., Whitehouse Station, NJ.
§Lescol®, Novartis Pharmaceuticals Corporation, East Hanover, NJ.
EDSS = Expanded Disability Status Scale; Gd+ = gadolinium-enhancing; MS = multiple sclerosis.

efficacy of IM IFNβ-1a (Avonex®, Biogen Idec, Inc.) in patients with RRMS. We also conducted studies to assess the impact of statins on interferon (IFN)-induced gene expression.

METHODS

Design. The methods for the SENTINEL study were described previously.[4] Patients from the IM IFNβ-1a monotherapy arm (n = 582) were divided into two groups: a statins group (n = 40) and a no-statins group (n = 542). The statins group patients took statins during the course of the study. Ethics committee approval and informed patient consent were obtained for the original SENTINEL study.[4] The present post hoc analysis did not involve interaction with patients, acquisition of new data, or identifying patients. Therefore, additional approval and informed consent was not requested.

Endpoints. Analyses were conducted on 2-year endpoints, including cumulative probability of sustained disability progression as defined in the SENTINEL study[4]; rate of clinical relapse; number of new or enlarging T2-hyperintense lesions; and number of gadolinium-enhancing (Gd+) lesions.[4] Safety data also were collected.

Biological response study. The biological effects of concomitant statins and IM IFNβ-1a administration compared with IM IFNβ-1a alone were assessed in patients with RRMS and in an in vitro model of the effects of statins on IFN-stimulated gene (ISG) expression.

ISG expression was determined in ≥30 patients with RRMS after their first dose of IM IFNβ-1a, and included one patient treated with atorvastatin (Lipitor®, Pfizer, Inc., New York, NY) 80 mg per day before initiation and during treatment with IM IFNβ-1a. Gene expression was determined using a customized cDNA macroarray assay.[5] RNA was isolated from blood samples 12 hours before and after the first IM IFNβ-1a injection. ISG induction ratio was defined as fold induction 12 hours after the first dose of IM IFNβ-1a relative to preinjection levels, normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) "housekeeping" gene.[6] For in vitro studies, HT1080 and Jurkat cells were incubated for 24 hours with 10 μM mevastatin (Sigma-Aldrich, St. Louis, MO) or left untreated, followed by the addition of recombinant IFNβ (1,000 U/mL) for a further 6 hours. Cells were harvested and RNA (10 μg) was analyzed for ISG expression by nuclease protection assay.[7,8]

To establish mevastatin activity in these cell culture systems, serum-starved HT1080 cells were treated with mevastatin at various doses (1, 5, or 10 μM) for 24 hours or left untreated. Cytoplasmic and membrane protein extracts were prepared[9] and probed with anti-Rac-1 antibodies. Incubation with a primary antibody was carried out at 4°C overnight and with a secondary antibody at 37°C for 1 hour. Immunoreactivity was visualized using the ECL Western blotting system (Amersham Biosciences/GE Healthcare, Chalfont St. Giles, UK). The membrane was stripped and reprobed with anti-β-actin antibodies to normalize protein loading.

Statistical analysis. The cumulative probability of sustained disability progression was estimated using the Kaplan-Meier method, and treatment effect was determined from a Cox proportional hazards model adjusted for baseline Expanded Disability Status Scale (EDSS) score and baseline number of Gd+ lesions. The mean change in EDSS score from baseline to 2 years was analyzed using an analysis of covariance (ANCOVA) model adjusted for baseline score and the number of relapses in the year before study entry. The annualized relapse rate was analyzed using Poisson regression adjusted for the number of relapses in the year before study entry, baseline EDSS score, baseline number of Gd+ lesions, and age. The number of Gd+ lesions was analyzed using a rank-based ANCOVA adjusted for baseline number of Gd+ lesions and age. The number of new or enlarging T2-hyperintense lesions was analyzed using a rank-based ANCOVA adjusted for baseline number of T2 lesions (<9 vs ≥9), baseline number of Gd+ lesions, and age.

RESULTS

Clinical study. ***Patients.*** Of the 582 patients randomized to IM IFNβ-1a monotherapy, 40 took at least one dose of statin (statins group) and 542 did not take any statins during the course of the study (no-statins group). Twenty patients were taking statins before randomization; statin therapy was started after the first dose of study medication in 19 patients, and one patient had an unknown statin start date. The median duration of concomitant IM IFNβ-1a and statin administration was 657 days (1.8 years). Most patients took atorvastatin (n = 26) or simvastatin (Zocor®, Merck & Co., Inc., Whitehouse Station, NJ) (n = 13), including four patients who took both throughout the course of the study (table 1). In general, patients were well matched at baseline for disease severity; however, patients in the

Table 2 Clinical efficacy outcomes at 2 years

Endpoint	No statins (n = 542)	Statins (n = 40)	*p* Value
Adjusted annualized relapse rate (95% CI)	0.67 (0.61, 0.74)	0.66 (0.46, 0.96)	0.937*
No. of relapses (%)			0.466†
0	198 (37)	17 (43)	
1	154 (28)	9 (23)	
2	94 (17)	8 (20)	
3	46 (8)	5 (13)	
≥4	50 (9)	1 (3)	
Cumulative probability of sustained disability progression‡ at 2 years	0.29	0.31	0.438§
Change in EDSS score from baseline, mean (SD)	0.31 (0.907)	0.22 (1.149)	0.716‖

*From Poisson regression with overdispersion, adjusted for the number of relapses in 1 year before study entry, baseline EDSS, baseline number of Gd+ lesions, and age.
†From chi-square test.
‡Defined as ≥1.0-point increase in EDSS score from a baseline score of ≥1.0 or a ≥1.5-point increase from a baseline score of 0, sustained for 12 weeks.
§From Cox proportional hazards model, adjusted for baseline EDSS and baseline number of Gd+ lesions.
‖From analysis of covariance, adjusted for baseline EDSS and the number of relapses in 1 year before study entry.
CI = confidence interval; EDSS = Expanded Disability Status Scale.

statins group were older than those in the no-statins group (44.0 vs 38.7 years).

Outcomes. No significant differences were observed between the statins group and the no-statins group on measures of clinical and MRI efficacy (tables 2 and 3). The adjusted annualized relapse rate at 2 years was similar in both groups ($p = 0.937$), as was the proportion of relapse-free patients and patients with ≥1 relapse ($p = 0.466$) and the cumulative probability of sustained disability progression (hazard ratio = 1.27 [95% confidence interval (CI) = 0.70, 2.29]; $p = 0.438$). The median number of Gd+ lesions was 0 for both groups after 1 year ($p = 0.927$) and 2 years ($p = 0.604$). Similar proportions of patients in both groups were free of Gd+ lesions at 2 years ($p = 0.788$). Over 2 years, the median number of new or enlarging T2-hyperintense lesions was one for the statins group compared with two in the no-statins group ($p = 0.802$).

A sensitivity analysis to determine the effect of uninterrupted therapy on measures of disease progression revealed that there were no significant differences between the 17 patients treated for 2 full years with concomitant statins and IM IFNβ-1a and the 438 patients treated for 2 full years with IM IFNβ-1a monotherapy in adjusted annualized relapse rate (0.59 no statins vs 0.67 statins; $p = 0.650$); disability progression (0.26 vs 0.18; $p = 0.689$); or median number of Gd+ lesions (0, both groups; $p = 0.997$) or T2-hyperintense lesions (2 vs 0; $p = 0.612$).

The most commonly reported adverse events in patients in the statins group were fatigue, headache, back pain, extremity pain, arthralgia, depression, and asthenia. For patients in the no-statins group, the most common adverse events were headache, nasopharyngitis, fatigue, back pain, arthralgia, and hypoesthesia (table e-1 on the *Neurology*® Web site at www.neurology.org). The incidence of muscle-related pain was higher for patients in the statins group than for patients in the no-statins group (back pain, 38% statins vs 27% no statins; extremity pain, 35% statins vs 20% no statins; myalgia, 18% statins vs 9% no statins). Patients in the statins group were more likely than patients in the no-statins group to experience a shift from baseline to high alanine transaminase, alkaline phosphatase, and aspartate transaminase levels (table e-1).

Biological response study. ***ISG expression analysis.*** Macroarray assays documented strong ISG expression (mean number of ISG induction ratios ≥2 = 57) in ≥30 patients with RRMS. One patient concurrently using atorvastatin 80 mg/day expressed 47 ISGs with induction ratio ≥2 (figure e-1).

In vitro effect of mevastatin on ISG expression. Pretreatment of HT1080 (a fibrosarcoma cell line) or Jurkat (a T-cell line) with mevastatin did not alter IFNβ-1a–induced expression of four well-characterized ISGs (TRAIL, β-R1, 9-27, and 6-16) (figure e-2). Mevastatin reduced the association of Rac-1 with cell membranes indicating biological activity despite lack of effect on ISG induction (figure e-3).

DISCUSSION In the current analysis of data from SENTINEL, there was no evidence that standard doses of statins used to treat hyperlipidemia reduced the efficacy of IM IFNβ-1a with respect to annualized relapse rates, sustained disability progression, or lesion burden. The adverse events experienced by patients in both groups were similar, although more patients in the statins group reported musculoskeletal pain and hepatic enzyme abnormalities. Additional studies demonstrated robust upregulation of ISGs ex vivo in a patient taking atorvastatin 80 mg at the time of the initial IM IFNβ-1a injection, equivalent to that observed in patients treated with IM IFNβ-1a alone. Mevastatin did not alter ISG induction in vitro, while Rac-1 translocation assays showed that the statin was biologically active in vitro.

These findings contrast with another study that found significantly greater risk of new and enhancing lesions or relapses in patients taking atorvastatin in combination with subcutaneous IFNβ-1a.[10] However, there were several methodologic differences between studies. Our study was a retrospective analysis of 2 years of treatment with IM IFNβ1-a, whereas

Table 3 MRI outcomes at 1 and 2 years

Endpoint	No statins (n = 542)	Statins (n = 40)	*p* Value
Median Gd+ lesions (min, max) n at year 1	0 (0, 43) 505	0 (0, 4) 39	0.927*
Gd+ lesions/patient, year 1, n (%)			0.842†
0	366 (72)	32 (82)	
1	68 (13)	5 (13)	
2	27 (5)	1 (3)	
≥3	44 (8)	1 (3)	
Median Gd+ lesions (min, max) n at year 2	0 (0, 41) 457	0 (0, 6) 36	0.604*
Gd+ lesions/patient, year 2, n (%)			0.788†
0	331 (72)	28 (78)	
1	58 (13)	6 (17)	
2	29 (6)	1 (3)	
≥3	39 (7)	1 (3)	
New or enlarging T2 lesions, years 0–1, median (min, max) n	1 (0, 28) 507	0 (0, 11) 39	0.751‡
T2 lesions/patient, years 0–1, n (%)			0.255†
0	228 (45)	20 (51)	
1	72 (14)	6 (15)	
2	59 (12)	7 (18)	
≥3	148 (27)	6 (15)	
New or enlarging T2 lesions, years 1–2, median (min, max), n	1 (0, 43) 458	0 (0, 10) 36	0.658‡
T2 lesions/patient, years 1–2, n (%)			0.509†
0	196 (43)	19 (53)	
1	52 (11)	6 (17)	
2	45 (10)	3 (8)	
≥3	165 (30)	8 (20)	
New or enlarging T2 lesions, years 0–2, median (min, max) n	2 (0, 64) 458	1 (0, 19) 36	0.802‡
T2 lesions/patient, years 0–2, n (%)			0.108†
0	141 (31)	14 (39)	
1	47 (10)	8 (22)	
2	47 (10)	1 (3)	
≥3	223 (41)	13 (33)	

*From rank-based analysis of covariance, adjusted for baseline number of Gd+ lesions and age.
†From Fisher exact test.
‡From rank-based analysis of covariance, adjusted for baseline number of T2 lesions (<9 vs ≥9), baseline number of Gd+ lesions, and age.
Gd+ = gadolinium-enhancing.

the other was a prospective study of 6 months of treatment with SC IFNβ1-a.[10] Patients in our study took five different statins at a variety of doses, most of which were ≤20 mg/day; patients assigned to statin treatment in the other study received atorvastatin 40 or 80 mg/day only.[10] Also, the number who received no statins in our study (n = 542) was larger than that in the other study (n = 9).[10]

Based on the results of the current study, we could discern no significant effect of statins at usual therapeutic doses on treatment with IM IFNβ-1a in patients with RRMS, and biological studies failed to demonstrate an effect of statins on the primary molecular response to IFNβ.

AUTHOR CONTRIBUTIONS

Statistical analysis for this manuscript was conducted by Amy Pace, ScD, Biogen Idec, Inc., Cambridge, MA.

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MEDICATIONS AND MEDICAL DEVICES

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