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Atorvastatin Combined To Interferon to Verify the Efficacy (ACTIVE) in relapsing–remitting active multiple sclerosis patients: a longitudinal controlled trial of combination therapy

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Abstract

A large body of evidence suggests that, besides their cholesterol-lowering effect, statins exert anti-inflammatory action. Consequently, statins may have therapeutic potential in immune-mediated disorders such as multiple sclerosis. Our objectives were to determine safety, tolerability and efficacy of low-dose atorvastatin plus high-dose interferon beta-1a in multiple sclerosis patients responding poorly to interferon beta-1a alone. Relapsing–remitting multiple sclerosis patients, aged 18–50 years, with contrast-enhanced lesions or relapses while on therapy with interferon beta-1a 44 μg (three times weekly) for 12 months, were randomized to combination therapy (interferon + atorvastatin 20 mg per day; group A) or interferon alone (group B) for 24 months. Patients underwent blood analysis and clinical assessment with the Expanded Disability Status Scale every 3 months, and brain gadolinium-enhanced magnetic resonance imaging at screening, and 12 and 24 months thereafter. Primary outcome measure was contrast-enhanced lesion number. Secondary outcome measures were number of relapses, EDSS variation and safety laboratory data. Forty-five patients were randomized to group A ($n = 21$) or B ($n = 24$). At 24 months, group A had significantly fewer contrast-enhanced lesions versus baseline ($p = 0.007$) and significantly fewer relapses versus the two pre-randomization years ($p < 0.001$). At survival analysis, the risk for a 1-point EDSS increase was slightly higher in group B than in group A ($p = 0.053$). Low-dose atorvastatin may be beneficial, as add-on therapy, in poor responders to high-dose interferon beta-1a alone.

Keywords

activity, combination therapy, interferon beta, multiple sclerosis, statin

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Introduction

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system, and one of the leading causes of neurological disability among young adults. Indeed, it affects approximately 1 million people world-wide,[1] with a median onset age of 28 years.[2] The drugs available for MS are immunomodulators, in particular compounds of the beta interferon (IFN) family. However, they are associated with incomplete efficacy and several adverse effects. Therefore, new, more efficacious and more easily administered drugs, or combination therapies, are eagerly awaited.

Atorvastatin, an HMG-CoA reductase inhibitor, widely used to reduce serum cholesterol levels, was found to decrease both the severity and number of relapses in an animal model of MS, that is, murine

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experimental autoimmune encephalomyelitis.[3,4] This finding prompted several trials with statins alone or as add-on therapy to IFN beta in relapsing–remitting (RR) MS patients. However, the trials produced contrasting results. Simvastatin, another HMG-CoA reductase inhibitor, reduced the numbers and size of contrast enhanced lesions (CELs) in brain magnetic resonance imaging (MRI) studies of RR-MS patients in an open label trial.[5] Atorvastatin, as add-on therapy to IFN beta-1a 44 μg subcutaneously (sc) three times weekly, resulted in increased disease activity in a small cohort of RR-MS patients in a double-blind placebo-controlled trial lasting 6 months.[6] On the other hand, Paul and colleagues[7] reported a beneficial effect of high-dose atorvastatin, alone or in combination with IFN, on the development of new CELs at MRI, over a 9-month treatment period, with a trend towards significance in the combination treatment group. Lastly, a retrospective study by Rudick et al.[8] comparing patients on IFN beta-l a 30 μg intramuscular (i.m.) therapy alone ($n = 542$) and patients on the same IFN schedule but taking various statins for hyperlipidaemia ($n = 40$), at different dosages and for different lengths of time, showed no clinical or MRI differences. In addition, statins did not affect either the *ex vivo* or *in vitro* induction of IFN-stimulated genes.[8]

The aim of this study was to investigate the safety and efficacy of low-dose atorvastatin as add-on therapy in a cohort of RR-MS patients responding poorly to IFN beta-1a alone, in long-term follow-up.

Patients and methods

From April 2005 to April 2006, 93 consecutive outpatients of the MS Center of the Federico II University Hospital (Naples, Italy), with clinically definite RR–MS according to the McDonald criteria,[9] aged 18–50 years, were treated with IFN beta-1a 44 μg three times weekly. Patients were monitored for 12 months (run-in period); visits were scheduled every 3 months and included Expanded Disability Status Scale (EDSS) assessment, and unscheduled visits to verify probable relapses each time the patients contacted the centre for any subjective symptom. Disease history, including information about relapses that occurred during the 24 months before randomization, was collected. At the end of the 12-month run-in period, a screening visit with EDSS assessment and MRI was scheduled. Patients with disease activity (at least one relapse or one gadolinium-enhanced lesion at screening MRI) were randomized to continue IFN in combination with atorvastatin 20 mg per day (group A) or IFN alone (group B) for 24 months.

After randomization, EDSS and laboratory parameters were assessed at 1 month and every 3 months for 24 months, and brain MRI was performed after 12 and 24 months. Both the examining neurologist (for EDSS and relapse assessment) and the neuroradiologist were blinded to treatment assignment. The primary outcome was number of MRI CELs. Secondary outcomes were the number of clinical relapses, EDSS score variation and safety laboratory parameters. During the study, patients received no other immunomodulatory or immunosuppressive therapy. In the case of a confirmed relapse, a 3-day cycle of methylprednisolone (totally 3 g intravenously (i.v.)) was prescribed.

Ethics committee approval was obtained, and each patient gave their signed informed consent to participate in the study. Atorvastatin was supplied by the manufacturer Pfizer (Pfizer Italia, Latina, Rome). Safety measures were as follows: patients underwent serum chemistry and haemocromocytometric analyses that included Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), kidney function tests, total cholesterol, LDL cholesterol, HDL cholesterol, creatine phospho-kinase (CPK), thyroid function tests and vital signs. Blood testing was performed in the morning after a 12-hour fast. Laboratory values 2.5-fold below or above normal range were considered adverse events.

Statistical analysis

Continuous variables were compared by mixed-model analysis of variance (ANOVA) with the F test. Categorical variables were compared by the χ^2 test with Fisher exact test, and, where appropriate, by non-parametric multivariate analysis of variance (MANOVA) for repeated measures (per-protocol analysis, performed only in patients who completed 24 months of treatment). For survival analyses, the time in the cohort was defined from study entry to the last clinical observation and/or failure event. Failure event was defined as a 1-point EDSS increase sustained for at least 3 months. We used the log-rank test to compare the equality of survival functions. For all statistical tests, a p-value < 0.05 was considered significant. The STATA 10 (StataCorp, College Station, TX, USA) program was used for statistical analyses.

Results

At screening, 45 subjects had active disease (23 had relapses and 22 had CELs while on IFN treatment) and were enrolled and randomized to combination therapy ($n = 21$; group A) or IFN alone ($n = 24$; group B). At baseline, age, sex distribution, disease duration, the total number of relapses (Table 1), the number of relapses during the 24 months before randomization, CEL number and EDSS score (Table 2) were comparable in the two groups. The number of

Table 1. Clinical and demographic characteristics at baseline of group A (interferon + atorvastatin) and group B (interferon)

	Group A atorvastatin + interferon beta alone	Group B atorvastatin + interferon beta alone	*p*
Number	21	24	
Age[a] (years)	31.5 ± 9	32.9 ± 7	0.81
Female/male	14/7	15/9	0.77
Disease duration[a] (months)	98.4 ± 68.1	104.2 ± 64.3	0.97
Total relapses[a]	6.9 ± 3.7	6.6 ± 4.3	0.52

[a]Mean ± SD.

CELs at screening ranged from 0 to 5 in group A and from 0 to 9 in group B. During follow-up, 2 patients dropped out in group A: the first at month 9 for depression and the second at month 18 for amenorrhoea and increased spasticity. Five patients of group B dropped out, 1 for pregnancy at month 12, 2 for clinical disease activity at month 15 and month 18 respectively, 1 for secondary progression and 1 for depression, both at month 21. Thirty-eight patients (19 group A and 19 group B) completed the study.

Primary endpoint – number of contrast-enhanced lesions. Treatment resulted in a decrease in the number of CELs in both groups. The difference between baseline and 24-month follow-up was significant in group A ($p = 0.007$) but not in group B ($p = 0.317$) (Table 2 and Figure 1). The difference between the two groups was not significant ($p = 0.10$).

Secondary endpoints – number of relapses, EDSS score variations and laboratory parameters. There were 10 relapses in 8 group A patients (53% relapse-free) and 30 relapses in 13 group B patients (32% relapse-free). The mean relapse number during the 24 months of follow-up was 0.5 ± 0.7 in group A versus 1.6 ± 1.8 in group B ($p = 0.03$). There were fewer relapses during the 24-month follow-up than during the 2 years before randomization in both groups; the difference was significant in group A ($p < 0.001$) but not in group B ($p = 0.33$) (Table 2; Figure 2), and there was a significant group effect ($p < 0.005$).

A sustained increase of EDSS score of at least 1 point was observed in 4 patients of group B (17%), 2 of whom dropped out before study completion, and in no patient of group A. ANOVA analysis with repeated measures showed a significant increase in EDSS at 24 months in group B patients ($p = 0.039$) but not in group A patients ($p = 0.80$) without a significant group effect ($p = 0.60$) (Table 2). We used survival analysis to determine the likelihood of experiencing a 1-point EDSS increase in the two groups. Group B patients were at a greater risk for clinical worsening than group A patients, but the difference was not quite significant ($p = 0.053$).

At baseline, serum levels of total, LDL and HDL cholesterol and triglycerides did not differ significantly between the two groups. In fact, total cholesterol ranged between 2.5 and 6.8 mmol/L in group A and between 2.7 and 7.6 mmol/L in group B (normal laboratory range: 2.4–5.5 mmol/L; desirable value: <4.9 mmol/L). LDL ranged between 1.1 and 5 mmol/L in group A and between 0.7 and 1.9 mmol/L in group B (normal laboratory range:1.5–3.9 mmol/L; desirable value: <3.4 mmol/L). Serum total cholesterol and LDL serum levels decreased significantly in group A from a mean of 4.2 ± 1.1 mmol/L and 2.2 ± 0.9 mmol/L respectively, at baseline, to a mean of 3.4 ± 1 mmol/L and 1.6 ± 0.8 mmol/L, at the 24-month follow-up examination ($p = 0.03$ and $p < 0.005$, respectively). The other laboratory parameters, even CPK and liver enzymes, remained unchanged. No muscle pain or cramps were reported in either group.

Table 2. Clinical and neuroradiologic characteristics of group A (interferon + atorvastatin) and group B (interferon) patients at baseline and after 24 months of follow-up

	Group A			Group B			Treatment effect at repeated measures analysis
	Baseline	Follow-up	*p*	Baseline	Follow-up	*p*	*p*
CELs[a]	1.3 ± 1.5	0.11 ± 0.45	$p = 0.007$	1.1 ± 2.3	0.6 ± 1.5	$p = 0.32$	$p = 0.10$
Relapses[a,b]	2.6 ± 1.1	0.5 ± 0.7	$p < 0.001$	2.1 ± 1.2	1.6 ± 1.8	$p = 0.33$	$p < 0.005$
EDSS[a]	2.8 ± 1.1	3.0 ± 1.1	$p = 0.80$	3.0 ± 1.1	3.4 ± 1.1	$p = 0.04$	$p = 0.60$

[a]Mean ± SD.
[b]Number of relapses in the 2 years before randomization.
CELs, contrast-enhanced lesions; EDSS, Expanded Disability Status Scale.

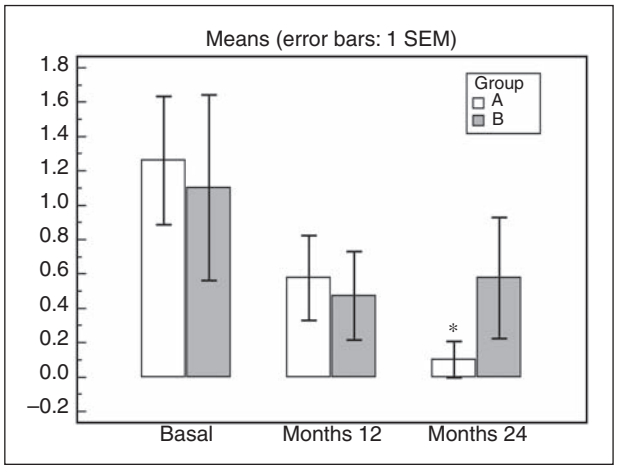

Figure 1. Contrast-enhanced lesions (means ± standard error of the mean) at baseline, and at 12 and 24 months of follow-up. Data are plotted for the two treatment groups. Group A: combination therapy; group B: interferon alone therapy. *$p = 0.007$ versus baseline.

Discussion

Several studies suggest that, besides their cholesterol-lowering effect, statins exert anti-inflammatory action. Therefore, these compounds may have therapeutic potential in immune-mediated disorders such as MS. Atorvastatin has been shown to modulate the clinical course of experimental autoimmune encephalitis, which is the best characterized animal (murine) disease model of MS.[3,4,10] Statins in combination with IFN beta exert synergistic effects in the suppression of T-cell proliferation.[11] On the other hand, lowering of cholesterol levels has been reported to have a detrimental effect in an animal model of the disease.[12]

Clinical trials have yielded contrasting results about the effect of statin/IFN combinations on RR-MS patients.[5–8] It is difficult to compare the data from these trials because of differences in the statins used, in the dosage and in the kinds of patients enrolled. In particular, Birnbaum and colleagues[6] reported that the addition of atorvastatin, 40 mg or 80 mg daily, to IFN beta-1a, 44 μg s.c. three times weekly, resulted in increased disease activity (at MRI and/or clinically). The authors suggested that this unexpected result was because, at the dosage used, statins could have exerted an antagonistic effect on IFN activity. On the other hand, no antagonistic effects emerged from the interim safety analysis of 47 patients in the SIMCOMBIN trial treated with simvastatin 80 mg or placebo in addition to IFN beta-1a 30 μg i.m.[13] Lastly, CEL number decreased in active RR-MS patients on atorvastatin 80 mg daily in combination with IFN beta-1a in a 9-month trial.[7]

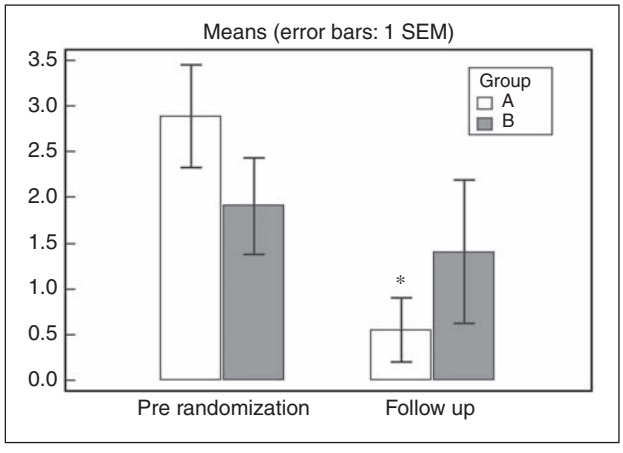

Figure 2. Relapses in the 2 years before randomization and in the 2 years of follow-up. Group A: combination therapy; group B: interferon alone therapy. *$p < 0.005$ compared versus pre-randomization.

We studied low-dose atorvastatin, as add-on therapy to IFN beta-1a, for the longest period reported so far, to determine whether it might be beneficial or detrimental, in an RR-MS population who responded poorly to IFN alone. Our results show that combination therapy was safe and well tolerated. More interestingly, we observed a significant reduction of CEL number at MRI and of relapses, with a stable EDSS score, only in the combination therapy group. Direct between-group comparison showed that the decrease in relapses was significantly associated to a treatment effect of statins, whereas there was a trend towards a decrease in CEL number. This discrepancy is probably due to the small sample sizes but, in any event, these results argue against a possible detrimental effect of the association, as also recently suggested.[6] Moreover, drop-outs because of disease activity or progression occurred only in the IFN-alone group. Regression to the mean, that is, a reduction of CEL number and relapses, is a spontaneous phenomenon in MS populations with active disease, irrespective

of therapy.[14] However, since our two groups were highly comparable at baseline, the different outcome of therapy observed at follow-up cannot be attributed solely to a regression to the mean effect.

In conclusion, our data suggest that a low dose of atorvastatin, as add-on therapy to IFN, is safe and might be beneficial in a population of RR-MS patients who respond poorly to IFN alone. Further studies, with larger cohorts of patients and a longer follow-up, are required to verify this affirmation and to clarify the effects of statins in MS.

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References

1. Baum H, Rothschild B. The incidence and prevalence of reported multiple sclerosis. *Ann Neurol* 1981; 10: 420–428.
2. Weinshenker B, Bass B, Rice G, et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. *Brain* 1989; 112: 133–146.
3. Aktas O, Waiczies S, Smorodchenko A, et al. Treatment of relapsing paralysis in experimental encephalomyelitis by targeting Th1 cells through atorvastatin. *J Exp Med* 2003; 197: 725–733.
4. Youssef S, Stuve O, Patarroyo JC, et al. The HMGCoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* 2002; 420: 78–84.
5. Vollmer T, Key L, Durkalski V, et al. Oral simvastatin treatment in relapsing–remitting multiple sclerosis. *Lancet* 2004; 363: 1607–1608.
6. Birnbaum G, Cree B, Altafullah I, Zinser M, Reder AT. Combining beta interferon and atorvastatin may increase disease activity in multiple sclerosis. *Neurology* 2008; 71: 1390–1395.
7. Paul F, Waiczies S, Wuerfel J, et al. Oral high-dose atorvastatin treatment in relapsing-remitting multiple sclerosis. *PLoS ONE* 2008; Apr 9: e1928.
8. Rudick RA, Pace A, Rani MR, et al. Effect of statins on clinical and molecular responses to intramuscular interferon beta-1a. *Neurology* 2009; 72: 1989–1993.
9. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; 50: 121–127.
10. Greenwood J, Walters CE, Pryce G, et al. Lovastatin inhibits brain endothelial cell Rho-mediated lymphocyte migration and attenuates experimental autoimmune encephalomyelitis. *FASEB J* 2003; 17: 905–907.
11. Neuhaus O, Strasser-Fuchs S, Fazekas F, et al. Statins as immunomodulators. *Neurology* 2002; 59: 990–997.
12. Miron V, Zehntner SP, Kuhlman T, et al. Statin therapy inhibits remyelination in the central nervous system. *Am J Pathol* 2009; 174: 1880–1890.
13. Sorensen PS, Frederiksen JL, Lycke L, et al. Does simvastatin antagonise the effect of interferon beta? Interim safety analysis of ongoing SIMCOMBIN study. *ECTRIMS 2007*, 11–14 October 2007, Prague, Czech Republic. Abstract 85.
14. Martìnez-Yèlamos S, Martìnez-Yèlamos A, Martìn Ozaeta G, et al. Regression to the mean in multiple sclerosis. *Mult Scler* 2006; 12: 826–829.