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Atorvastatin decreases high-sensitivity C-reactive protein in multiple sclerosis

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The anti-inflammatory potential of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, as reflected by modulation of C-reactive protein (CRP), might be beneficial in the treatment of patients with multiple sclerosis (MS). We evaluated serum levels of high-sensitivity (hs)-CRP in relapsing–remitting MS patients receiving interferon-β 1b and atorvastatin as add-on therapy. This study shows that interferon-β treatment is associated with increased serum levels of hs-CRP in MS patients ($P < 0.01$). In contrast, when atorvastatin is added to interferon-β, hs-CRP serum levels decrease to the normal range ($P < 0.05$), indicating an anti-inflammatory action of atorvastatin in MS. However, whether add-on treatment with atorvastatin modifies the course of MS remains to be investigated. *Multiple Sclerosis* 2008; **14:** 981–984. http://msj.sagepub.com

Key words: anti-inflammatory; atorvastatin; C-reactive protein; multiple sclerosis

Introduction

Statins are approved as cholesterol-lowering drugs and inhibit the 3-hydroxy-3-methylglutaryl coenzyme A reductase to reduce cholesterol biosynthesis. In addition, there is evidence for immunomodulatory and anti-inflammatory properties of statins, which may be favorable in the treatment of autoimmune disorders such as multiple sclerosis (MS).

Serum levels of high-sensitivity C-reactive protein (hs-CRP) were shown to reflect systemic low-grade inflammatory activity [1]. CRP is an acute-phase protein synthesized in the liver and is induced by cytokines such as interleukin-6 (IL-6), IL-1, and tumor-necrosis factor-α (TNF-α). In turn, CRP induces the synthesis of cytokines, cell adhesion molecules and tissue factor in peripheral blood mononuclear cells (PBMCs) and endothelial cells *in vitro* [2].

Although similar hs-CRP serum levels are detected in relapsing–remitting MS (RR-MS) patients and healthy controls, levels increase during clinical exacerbations of MS [3,4]. Elevated hs-CRP serum levels are associated with a higher risk for disability and progression in RR-MS, rendering hs-CRP as a potential surrogate marker of subclinical inflammatory activity and prognosis in MS [4]. Anti-inflammatory effects mediated by statins include the reduction of pro-inflammatory mediators such as CRP, serum amyloid A (SAA), cytokines, and cell adhesion molecules [5].

In this study, we determined the effects of interferon-β and atorvastatin as add-on therapy on hs-CRP serum levels in RR-MS patients.

Patients and methods

Patients

A total of 80 patients were enrolled in the Swiss Atorvastatin and Betaferon in Multiple Sclerosis (SWABIMS) trial. Patients were pretreated with interferon-β 1b (250 μg eod s.c) for 3 months and then randomized for either i) continuing with a interferon-β monotherapy ($n = 12$) or ii) combining interferon-β and atorvastatin (40 mg per os; $n = 16$). This substudy was approved by the Bernese Cantonal Ethical Review Board (KEK 17/05).

The primary outcome measures were the proportion of patients with new T2 lesions after 15 months of treatment. Secondary endpoints were Gadolinium (Gd)-enhancing lesions on T1-weighted images, clinical disease progression, and relapse rate. Inclusion criteria were diagnosis of RR-MS based on McDonalds criteria, disease duration ≥3 months, Expanded Disability Status Scale

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(EDSS) score from 0 to 3.5 at baseline, and at least one relapse in the past 2 years. In case of relapse, a 1-month interval from the last relapse and/or prednisone treatment to baseline was required. Median EDSS at baseline was 2.0 (range 0–3.5). In addition, serum samples were taken from eight healthy controls (mean age 34.0 years, range 22.1–54.4) who gave their informed consent.

Laboratory study

Peripheral venous blood from 28 MS patients (mean age 33.4 years, range 18.9–50.6) were taken at baseline and 3, 6, and 9 months later. Serum was obtained by standard centrifugation procedures and aliquots stored at −70 °C. CRP was determined in serum samples with an hs-CRP ELISA Kit (Alpha Diagnostic Int., San Antonio, Texas, USA) according to the manufacturers instructions (detection limit 0.35 ng/mL.) Samples were diluted 1:100 and studied in duplets. A re-measurement with a dilution of 1:200 was performed for samples out of range.

Statistical analysis

Statistical testing was performed with GraphPad Prism 5.0 (GraphPad, San Diego, California). The comparison between healthy controls and MS patients at baseline and the two treatment groups at month 3, 6, and 9 were done with a Mann–Whitney *U*-Test. Changes over time were evaluated with a Wilcoxon signed-rank test. A value of $P < 0.05$ was considered as statistically significant.

Results

Hs-CRP levels were similar in MS patients at baseline and in healthy controls. Treatment with interferon-β in MS patients over a period of 3 months was associated with an increase of hs-CRP serum levels as compared with hs-CRP levels at baseline ($P < 0.01$). This increase was as much as 37.0% (Figure 1A).

MS patients treated with interferon-β monotherapy had continuously elevated hs-CRP serum levels from month 3 to 9 (Figure 1B). In contrast, in MS patients randomized to interferon-β and atorvastatin as add-on therapy, decreased levels of hs-CRP were detected at month 6 and month 9, as compared with month 3, when they only received interferon-β ($P < 0.05$ (–44.0%) and $P < 0.05$ (–50.6%), respectively) (Figure 1B). The hs-CRP–lowering effect of atorvastatin was already present at month 6, after 3 months of atorvastatin treatment, when testing was performed versus patients continuing with a monotherapy at month 6 (Figure 1B; $P < 0.05$). Mean hs-CRP levels at month 3 before randomization did not show statistical differences between the patients continuing with a mono- versus combination therapy.

Discussion

The present study evaluated hs-CRP serum levels in RR-MS patients before initiation of immunomodulatory treatment, after a monotherapy with interferon-β, and a combination therapy of interferon-β and atorvastatin. The findings corrobo-

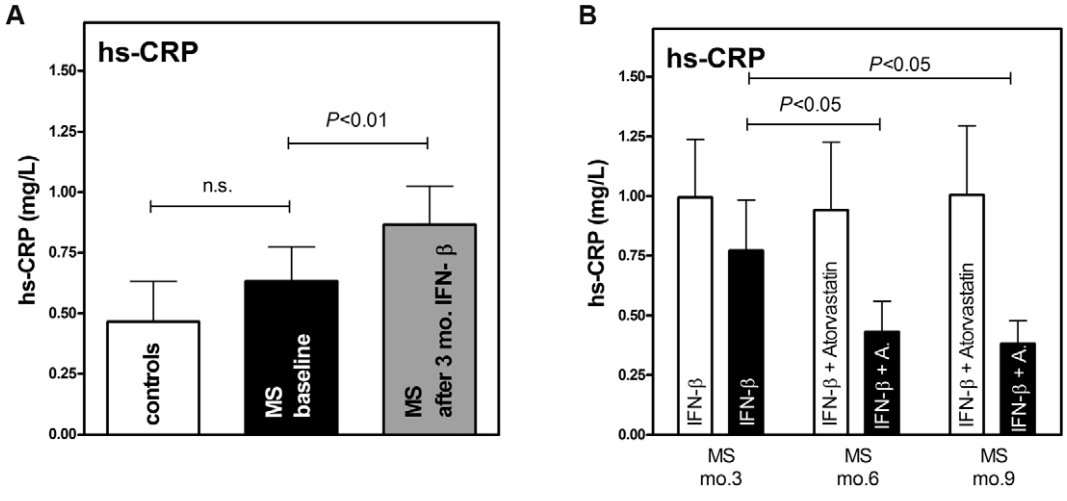

Figure 1 Serum levels of hs-CRP levels in healthy controls ($n = 8$) and 28 RR-MS patients participating in the Swiss Atorvastatin and Betaferon in Multiple Sclerosis (SWABIMS) study. Patients were pretreated with interferon-β for 3 months and were then randomized for continuing i) a monotherapy with interferon-β ($n = 12$) or ii) a combination therapy of interferon-β and atorvastatin ($n = 16$). Data are presented as mean ± SEM (standard error of mean). Abbrevations: interferon-β (IFN-β), atorvastatin (A).

rate results of previous studies reporting similar CRP or hs-CRP levels in MS patients and healthy controls [3,4,6]. Interferon-β treatment elevated hs-CRP serum levels significantly, and the increase of hs-CRP levels sustained over the observation period of 9 months. On the contrary, addition of atorvastatin reversed the interferon-β–induced increase of hs-CRP to the range of healthy controls. This effect is likely to display an aspect of anti-inflammatory action associated with statin treatment.

Increased circulating levels of hs-CRP were reported in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease, and heart transplantation [7]. In MS, only limited knowledge is available for hs-CRP in serum with regard to natural changes over time, interference with other drugs, and impact on the disease course. Clinical exacerbations and Gadolinium-enhancement on brain MRI were associated with a serum CRP increase in some but not all studies [3,4,8]. A transient increase of serum inflammatory markers such as CRP, SAA, β2-microglobulin, and neopterin for 24–72 h was reported after a single intramuscular injection of interferon-β 1a [6]. In the present study, RR-MS patients received s.c. injections every other day, which may have caused the sustained hs-CRP increase. However, it was reported that interferon-β 1a given three times per week s.c. lowered hs-CRP level in a dose-dependent manner even below the range of healthy controls [4]. Whether the modulation of CRP reported in serum following interferon-β treatment is induced by the local inflammatory reaction at the injection site or by direct interaction of interferon-β with hepatocytes is currently not known. IL-6, a cytokine with pro- and anti-inflammatory potential induces CRP by activation of the transcription factor STAT3 (signal transducer and activator of transcription 3), and increased production of IL-6 is directly related to flu-like symptoms after interferon-β injection [9,10]. Moreover, increased serum IL-6 levels at 10 h after interferon-β 1b s.c. application correlate with favorable treatment response and slower disease progression in Japanese MS patients, providing further evidence for the involvement of the IL-6/CRP axis in MS [11].

The molecular mechanisms underlying the anti-inflammatory action of statins were shown to be independent of their cholesterol-lowering activity. The CRP lowering property is more prominent for atorvastatin than for simvastatin or pravastatin [12]. Whether our observation on modulation of serum hs-CRP reflects a prophylactic downregulation of subclinical inflammatory activity with an aim to weaken the immunological cascade leading to autoimmune responses remains unclear. Statins inhibit interferon-β signalling in PBMC *in vitro* by deactivating STAT 1 [13]. STAT 3 and its family members contribute to the transcriptional activation of the CRP-gene induced by IL-6 [14]. Thus, it is conceivable that the sustained low-level activation of CRP induced by interferon-β monotherapy is antagonized by atorvastatin as add-on therapy. However, this does not necessarily imply that all effects of interferon-β are reversed by atorvastatin as add-on therapy.

Our study showed novel aspects on the pleiotropic effects of interferon-β and statins in MS. We provide evidence that interferon-β treatment is associated with a sustained increase of hs-CRP in serum, whereas add-on treatment with atorvastatin lowers hs-CRP. This anti-inflammatory effect of statins in MS may be favorable for the treatment of MS, but the clinical efficacy remains to be proven.

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References

1. Gabay, C, Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; **340**: 448–454.
2. Pasceri, V, Willerson, JT, Yeh, ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000; **102**: 2165–2168.
3. Giovannoni, G, Miller, DH, Losseff, NA, *et al*. Serum inflammatory markers and clinical/MRI markers of disease progression in multiple sclerosis. *J Neurol* 2001; **248**: 487–495.
4. Soilu-Hanninen, M, Koskinen, JO, Laaksonen, M, Hanninen, A, Lilius, EM, Waris, M. High sensitivity measurement of CRP and disease progression in multiple sclerosis. *Neurology* 2005; **65**: 153–155.
5. Abeles, AM, Pillinger, MH. Statins as antiinflammatory and immunomodulatory agents: a future in rheumatologic therapy. *Arthritis Rheum* 2006; **54**: 393–407.
6. Boylan, MT, Crockard, AD, Duddy, ME, Armstrong, MA, McMillan, SA, Hawkins, SA. Interferon-beta1a administration results in a transient increase of serum amyloid A protein and C-reactive protein: comparison with other markers of inflammation. *Immunol Lett* 2001; **75**: 191–197.
7. Osman, R, L'Allier, PL, Elgharib, N, Tardif, JC. Critical appraisal of C-reactive protein throughout the spectrum of cardiovascular disease. *Vasc Health Risk Manag* 2006; **2**: 221–237.
8. Giovannoni, G, Thorpe, JW, Kidd, D, *et al*. Soluble E-selectin in multiple sclerosis: raised concentrations in patients with primary progressive disease. *J Neurol Neurosurg Psychiatry* 1996; **60**: 20–26.
9. Nicoletti, F, Di Marco, R, Patti, F, *et al*. Short-term treatment of relapsing remitting multiple sclerosis patients with interferon (IFN)-beta1B transiently increases the blood levels of interleukin (IL)-6, IL-10 and IFN-gamma

without significantly modifying those of IL-1beta, IL-2, IL-4 and tumour necrosis factor-alpha. *Cytokine* 2000; **12**: 682–687.
10. Montalban, X, Duran, I, Rio, J, Saez-Torres, I, Tintore, M, Martinez-Caceres, EM. Can we predict flu-like symptoms in patients with multiple sclerosis treated with interferon-beta. *J Neurol* 2000; **247**: 259–262.
11. Nakatsuji, Y, Nakano, M, Moriya, M, *et al*. Beneficial effect of interferon-beta treatment in patients with multiple sclerosis is associated with transient increase in serum IL-6 level in response to interferon-beta injection. *Cytokine* 2006; **36**: 69–74.
12. Kwak, BR, Mulhaupt, F, Mach, F. Atherosclerosis: anti-inflammatory and immunomodulatory activities of statins. *Autoimmun Rev*. 2003; **2**: 332–338.
13. Dhawan, N, Reder, A. Statins block interferon signaling in human immune cells: potential loss of the therapeutic effect of IFN beta in multiple sclerosis. *Neurology*. 2007; **68**(Suppl 1): A364.
14. Zhang, D, Sun, M, Samols, D, Kushner, I. STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. *J Biol Chem*. 1996; **271**: 9503–9509.