

Atorvastatin Reduces Macrophage Accumulation in Atherosclerotic Plaques

A Comparison of a Nonstatin-Based Regimen in Patients Undergoing Carotid Endarterectomy

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Background and Purpose—The object of our study was to compare the effect of high-dose vs low-dose atorvastatin vs nonstatin-based treatment (cholestyramine plus sitosterol) on cell composition of carotid plaque.

Methods—We recruited 60 hypercholesterolemic patients (total cholesterol, 5.83–7.64 mmol/L) eligible for carotid endarterectomy. Three months before surgery, patients were randomized into 3 groups (n=20) receiving atorvastatin 10 mg/day (AT-10) or atorvastatin 80 mg/day (AT-80) or cholestyramine 8 g/day plus sitosterol 2.5 g/day. Analysis of cell composition was performed on endarterectomy specimens.

Results—The 3 treatments resulted in a significant reduction of total cholesterol and low-density lipoprotein cholesterol (LDL-C), although the decrease in total cholesterol and LDL-C was of smaller magnitude in the cholestyramine plus sitosterol group. The 3 regimens did not influence the levels of inflammatory markers (including high-sensitivity C-reactive protein). Macrophage content was significantly lower in the AT-10 group plaques compared to the cholestyramine plus sitosterol group. It was further reduced in the AT-80 group plaques. These differences were no longer significant after adjustment for changes in LDL-C. No difference in lymphocyte number was observed among treatments, whereas the content of smooth muscle cells was higher in the AT-80 group. An inverse association was observed between LDL-C changes in the 3 groups and macrophage content in the plaques.

Conclusions—Short-term treatment with high-dose statin is superior to a nonstatin lipid-lowering regimen in reducing the macrophage cell content within atherosclerotic lesions, but this effect was determined by the degree of LDL-C-lowering. (*Stroke*. 2010;41:1163-1168.)

Key Words: atherosclerosis ■ carotid artery ■ lipids ■ lipoprotein ■ macrophages ■ statins

The impact on cardiovascular events achieved by statin therapy seems to be mostly attributable to the cholesterol-lowering effect, with a highly debated contribution of the lipid-independent pleiotropic effects.^{1–4} However, a short-term benefit has been documented for patients treated with statins in acute coronary syndromes^{4,5} and other clinical settings.⁶ These observations strengthened the hypothesis of additional so-called pleiotropic actions of statins.⁷ For instance, several clinical studies demonstrated an anti-inflammatory effect of treatment with statins (such as C-reactive protein-lowering)^{3,8–10} that would represent the likely explanation for a further benefit attributable to this class of drugs. However, there is evidence that low-density lipoprotein cholesterol (LDL-C)-lowering per se might con-

tribute to the reduction of inflammatory biomarkers in statin-treated patients.¹¹ In addition, we do not know how different lipid-lowering regimens (ie, statins vs nonstatin and intensive vs moderate therapy) can modulate the inflammatory burden within atherosclerotic lesions. A previous study by Crisby et al¹² that was performed in 10 patients undergoing carotid endarterectomy after a 3-month pravastatin treatment showed a lower macrophage and lymphocyte content of the plaque, along with a reduced accumulation of lipids compared to arteries from control subjects. However, a control group composed of patients treated with nonstatin LDL-C-lowering therapy was not included in this study. Intensive as compared to moderate statin therapy has been proven to be superior in improving cardiovascular outcome in clinical trials,¹³

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whereas data are lacking on the benefits on plaque cellular composition of such an intensive approach.

We therefore sought to investigate how different lipid-lowering strategies (nonstatin therapy, low-dose statin, and high-dose statin) affect cellular composition of carotid plaque over a short-term period of 3 months. Specifically, we tried to dissect the LDL-C-lowering impact on plaque cellular composition as compared to the lipid-independent contribution on plaque macrophage and smooth muscle cells.

Subjects and Methods

Study Design

Sixty hypercholesterolemic patients (total cholesterol [TC] range, 5.83–7.64 mmol/L) never treated with lipid-lowering drugs, with symptomatic carotid stenosis $\geq 70\%$ (NASCET criteria),¹⁴ and therefore were eligible for carotid endarterectomy were recruited in 3 different study centers. All patients have been enrolled within 20 to 30 days from the clinical event and randomized to 1 of 3 treatment groups. Each group composed of 20 patients received atorvastatin 10 mg/day (AT-10 group) or atorvastatin 80 mg/day (AT-80 group) or cholestyramine (Questran, Bristol Myer Squibb) 8 g/day plus sitosterol (Unilever) 2.5 g/day (C-S group) for 3 months before the vascular procedure. Patients underwent carotid endarterectomy after 12 weeks plus or minus 2 days from the beginning of the active therapy. A placebo group was not included for ethical reasons because of the high cardiovascular risk profile in this population.

The study was approved by the local Ethics Committee and registered with ClinicalTrials.org (CT Identifier: NCT01053065). All patients gave informed consent.

Blood Samples Analysis

At the beginning of the study and at surgery as well, blood samples were collected to assess the lipid profile (TC, LDL-C, high-density lipoprotein cholesterol, triglycerides), level of inflammatory markers (high-sensitivity C-reactive protein, IL-6, IL-8, IL-10, IL-1 β , RANTES, monocyte chemoattractant protein-1, tumor necrosis factor- α , sCD40L), and adhesion molecules (soluble-P selectin, soluble vascular cell adhesion molecule-1 [sVCAM-1]). Serum levels of IL-6, IL-8, IL-1 β , IL-10, and tumor necrosis factor- α were determined by chemiluminescent immunometric assay on the Immulite 1000 analyser (IMMULITE; Siemens Diagnostics). Soluble P-selectin, sCD40L, monocyte chemoattractant protein-1, sVCAM-1, and RANTES concentrations were measured by enzyme-linked immunosorbent assay (BioSource International). Nephelometry was used for the quantitative determination of serum C-reactive protein and C3 and C4 levels by using a Behring nephelometer analyzer (Dade-Behring).

Determination of Cellular Composition and Lipid Content of Carotid Plaques

Immediately after surgery, the endarterectomy specimens were snap-frozen in liquid nitrogen, embedded in OCT (Sakura), and stored at -80°C . Serial sections were taken at 8- μm intervals and processed for immunocytochemistry as previously described.¹⁵ The following monoclonal antibodies were used to determine the cellular composition of the lesions: SM-E7 anti-smooth muscle (SM) myosin heavy chains, HAM-56 antimacrophage (Dako), and CD45RO antilymphocyte (Dako). The SM-E7 reacts with SM-type myosin heavy chains (both SM1 and SM2) exclusively and recognizes all cells in the SM lineage.¹⁶ Primary antibodies (except for CD45RO) were applied to freshly cut unfixed cryosections (8- μm -thick). The controls for indirect immunocytochemistry were mouse nonimmune IgG rather than primary antibody and the secondary antibody alone. Nuclei were revealed with the use of hematoxylin and eosin staining in adjacent sections. A standard protocol of Sudan black staining was performed to establish the lipid content of the plaques.

Image Analysis of Sections From Endarterectomy Specimens

Digital images of the stained lesions were obtained using a Qwin digital camera (Leica) for image analysis.¹⁷ According to a method previously validated^{15,17} for each antibody, cell composition was assessed in 3 sections per specimen and 3 standard microscopic fields per section, excluding the media layer underneath the external elastic lamina and, when present, areas of nonspecific staining. Positive staining to the various antibodies was expressed as percentage of the total area. Total cellularity of the plaque was established in adjacent sections by counting hematoxylin positive nuclei. Areas positive for each antibody were adjusted for cellularity of the plaque.

The lipid content in the lesions was assessed as Sudan black-positive area and expressed as percentage of total plaque area. Analyses were performed independently by 2 investigators blinded to the treatments.

Statistical Analysis

Continuous variables were averaged and expressed as mean $\pm$ standard deviation. Subjects were compared by analysis of variance and Bonferroni correction. Positive areas for the different cell types were analyzed by analysis of covariance after correction for total cellularity of the sections. $P < 0.05$ was considered significant. SYSTAT version 10.0 (SPSS) package was used for this purpose.

Results

Baseline Population Characteristics and Effect of the Treatments on Lipid Profile

Patients in the 3 groups did not differ in terms of degree of carotid artery narrowing, age, gender, blood pressure, glycaemia, and plasma lipid levels (Table). All patients were using antiplatelets drugs (ie, aspirin or ticlopidine). The 3 treatments resulted in a significant reduction of TC, LDL-C, and nonhigh-density lipoprotein cholesterol after the 3-month period (Table). Whereas no significant differences in TC and LDL-C changes were observed between the AT-10 and AT-80 groups, the decrease in TC and LDL-C was of significantly smaller magnitude in the C-S group as compared to both AT-10 ($P < 0.0005$) and AT-80 ($P < 0.0005$). A similar and significant trend was seen for the nonhigh-density lipoprotein cholesterol, with a smaller effect in the C-S group. At the end of the study period, high-density lipoprotein cholesterol and triglyceride levels were not different among the 3 groups. We did not record any clinically significant side effect or major adverse event in any of the treatment groups.

Effect of the Treatments on Circulating Markers of Inflammation

The levels of high-sensitivity C-reactive protein were comparable across the 3 groups at baseline (AT-10, 4.72 ± 3.90 mg/L; AT-80, 2.87 ± 3.03 mg/L; C-S, 3.39 ± 2.05 mg/L) and at the end of the study (AT-10, 2.87 ± 2.62 mg/L; AT-80, 2.21 ± 2.52 mg/L; C-S, 2.73 ± 4.47 mg/L). The 3 regimens did not significantly affect the levels of the various circulating proinflammatory cytokines (including IL-6, IL-8, tumor necrosis factor- α ; data not shown). Other markers of inflammation such as RANTES or levels of complement components (c3–c4) were not affected.

Cellular and Morphometric Features of Carotid Plaques

Carotid endarterectomy specimens retrieved at surgery showed a significantly lower macrophage accumulation in plaques from

Table. Baseline Demographic Data and Lipids Profile Among the Study

Variables	Sitosterol+ Cholestyramine (n=20)	Atorvastatin 10 mg (n=20)	Atorvastatin 80 mg (n=20)	P
Age, yr	78.7±5.3	78.4±5.1	79.0±5.0	NS (0.930)
Male	11	7	9	NS (0.522)
Systolic blood pressure, mm Hg	142±16	142±15	146±17	NS (0.609)
Diastolic blood pressure, mm Hg	81±12	83±12	79±11	NS (0.581)
hs-CRP T1, mg/L	3.34±2.00	3.59±2.39	2.33±1.68	NS (0.145)
Total cholesterol T1, mmol/L	6.81±0.44	7.04±0.26	6.94±1.09	NS (0.667)
Total cholesterol T2, mmol/L	6.24±0.73	5.72±0.85	5.31±0.78	0.002
Δ Total cholesterol, mmol/L	−0.60±0.47	−1.40±0.49	−1.66±0.60	<0.0005
LDL-C T1, mmol/L	4.90±0.54	5.21±0.75	4.90±1.43	NS (0.577)
LDL-C T2, mmol/L	4.45±0.67	3.99±0.88	3.39±0.91	0.001
Δ LDL-C, mmol/L	−0.39±0.34	−1.30±0.65	−1.50±0.75	<0.0005
HDL-C T1, mmol/L	1.24±0.28	1.19±0.26	1.48±0.60	NS (0.071)
HDL-C T2, mmol/L	1.24±0.18	1.17±0.10	1.40±0.49	NS (0.115)
Δ HDL-C, mmol/L	0.01±0.14	0.20±0.11	−0.10±0.17	0.034
Non-HDL-C T1, mmol/L	5.57±0.52	5.88±0.80	5.46±1.53	NS (0.458)
Non-HDL-C T2, mmol/L	5.00±0.78	4.56±0.85	3.91±1.09	0.003
Δ Non-HDL-C, mmol/L	−0.62±0.49	−1.42±0.52	−1.55±0.65	<0.0005
Triglycerides T1, mmol/L	1.62±0.88	1.49±0.70	1.31±0.53	NS (0.423)
Triglycerides T2, mmol/L	1.33±0.43	1.28±0.24	1.31±0.44	NS (0.904)
Δ Triglycerides, mmol/L	−0.25±0.55	−0.28±0.70	−0.0±0.23	NS (0.209)

Continuous variables are reported as mean±SD.

HDL-C indicates high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; NS, not significant; T1, baseline; T2, follow-up; Δ, delta (T2 value−T1 value).

Pearson χ^2 for categorical variables and analysis of variance for continuous variables.

the AT-10 group, and even more were retrieved from the AT-80 group compared to the C-S group (Figures 1 and 2A). An opposite trend was observed for the atherosclerotic plaque SM cell content. A higher number of SM cells was detected in specimens from the AT-10 and AT-80 vs C-S groups, with significant difference between AT-80 and C-S groups (Figures 1 and 2A). Considering the significantly different impact of the 3 lipid-lowering regimens on LDL-C level, we adjusted

the analysis for both on treatment LDL-C levels and adjusted for changes in LDL-C. After adjusting for those using treatment LDL-C, macrophage content was still significantly lower in the AT-80 compared to the C-S groups (Figure 2B). Lower macrophage content and higher SM cell concentration was still observed, although not significantly, after adjustment for changes in LDL-C in the 3 groups (Supplemental Figure I, available online at <http://stroke.ahajournals.org>)).

Cellular features of carotid plaques

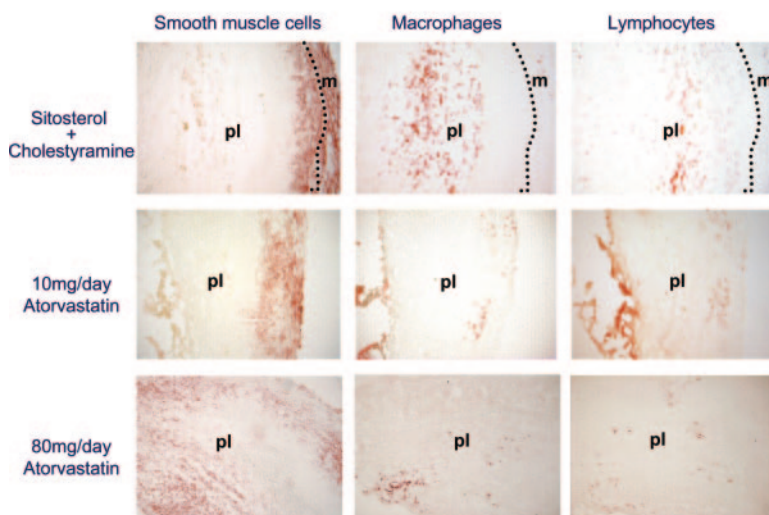


Figure 1. Cellular features of carotid plaques after 3 months of treatment. Representative figures of immunocytochemical staining for macrophages (HAM-56), SM cells (SM-E7), and lymphocytes (CD-45RO) in carotid plaques from the 3 groups of treatment. The dotted line defines the borderline between the plaque (pl) and the underlying media (m), when present. Magnification 100×.

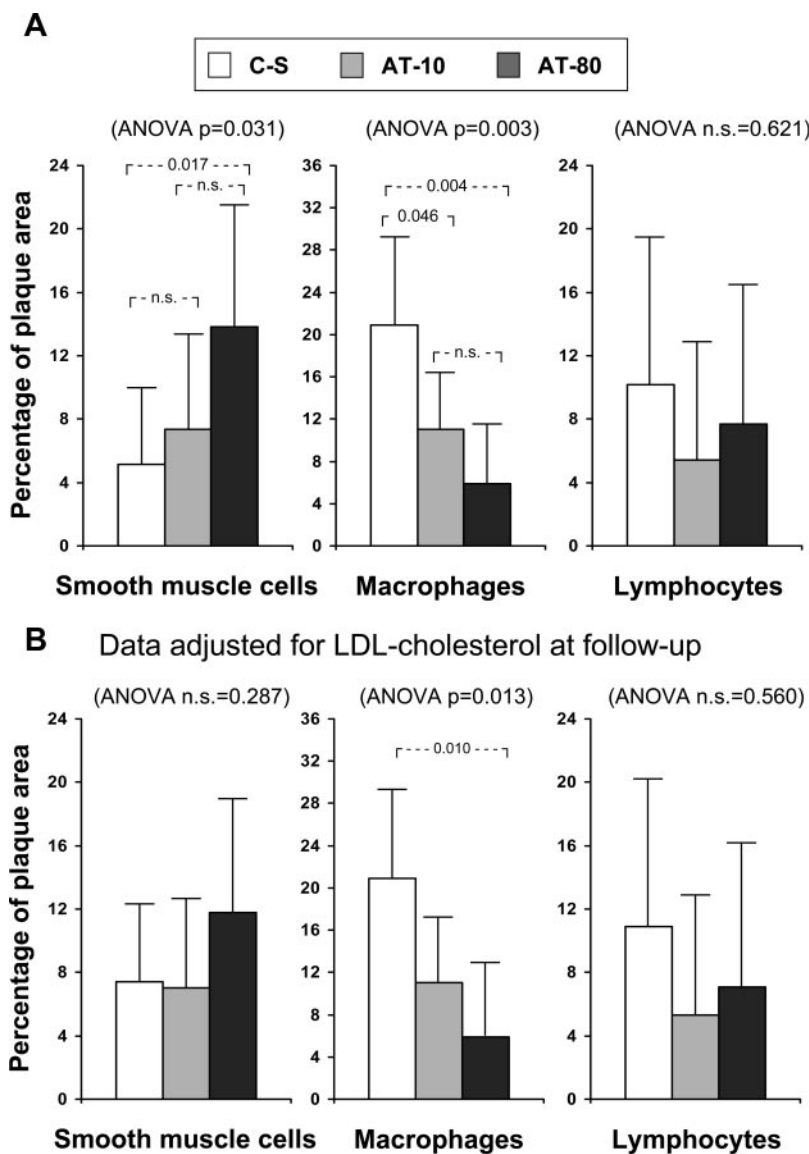


Figure 2. Cellular content of carotid plaques after 3 months of treatment. A, Atherosclerotic plaques obtained from patients treated with atorvastatin 10 mg/day (AT-10) showed a significantly lower content in macrophages compared to lesions from patients treated with cholestyramine plus sitosterol (C-S). Even lower macrophage accumulation was documented in plaques obtained from the patients treated with atorvastatin 80 mg/day (AT-80). No significant differences were observed in lymphocyte content among the 3 treatments, although smooth muscle cells were more abundant in plaques from the AT-80 group. Cellularity is expressed as percent of plaque area positive for specific antibody normalized for nuclei. B, Analysis of cellular content of carotid plaques adjusted for on-treatment LDL-C levels. After adjusting for on-treatment LDL-C, macrophage content was still significantly lower in the AT-80 compared to the C-S group.

Lymphocyte plaque concentration was similar in the 3 groups and it was not significantly affected by the active treatments. The lipid content of the atherosclerotic plaques was similar in the 3 groups (% of plaque area: C-S, 35 ± 16 ; AT-10, 37 ± 25 ; AT-80, 28 ± 19).

By linear regression analysis, a significant inverse association was observed between LDL-C changes observed in the 3 groups and macrophage content in the atherosclerotic plaques ($r = -0.456$; $P = 0.007$; Figure 3). The association between changes in LDL-C and SM cell content in the plaques showed a positive, although not significant, trend (Figure 3).

Discussion

In the present study, we demonstrated for the first time to our knowledge that a short-term treatment with statin is superior to a nonstatin lipid-lowering regimen in reducing the macrophage cell content inside atherosclerotic lesions, and this effect is, to a significant extent, modulated by the LDL-C changes. As expected, the highest reduction in TC and

LDL-C was found in the AT-80 group. This was accompanied by the most relevant impact in terms of remodeling of cell populations inside the plaque. The MIRACL study demonstrated that early, intensive treatment with atorvastatin 80 mg/day can reduce the risk of recurrent ischemic events in patients with acute coronary syndrome after 4 months of therapy.⁴ Our finding that treatment with atorvastatin 80 mg/day promoted the greatest reduction in macrophage content of plaques suggests a dose-dependent LDL-C–modulated effect of atorvastatin. This could represent a valid pathophysiological explanation for the beneficial effect observed in the MIRACL trial. Macrophage activation products, such as metalloproteinases, reactive oxygen species, and the like, are known to jeopardize the integrity of the fibrous cap by increasing the risk of plaque rupture. Therefore, it seems likely that reducing the number of macrophages in the lesion with statins can represent an important factor promoting plaque integrity. Finally, in agreement with the only previous report in humans,¹² we observed an increase in the number of SM cells in the plaque, at least in patients treated with the

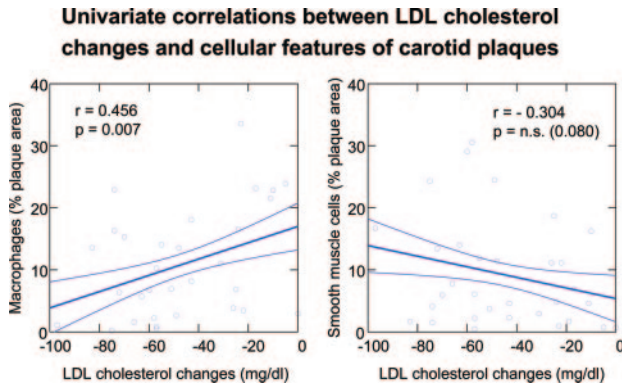


Figure 3. Univariate correlations between LDL-C changes in the 3 groups and cellular features of carotid plaques. Cholesterol changes were calculated as follow-up value minus baseline value. Cellularity is expressed as percent of plaque area positive for specific antibody normalized for nuclei. Left panel shows a progressive decline in macrophages as LDL-C level decreases. Right panel shows the increase in SM cells as LDL-C level decreases.

highest statin dose, namely the AT-80 group. This again suggests that a more stable plaque phenotype is the result of statin treatment, even in the short term. A recent study of the use of statin therapy in patients undergoing vascular surgery supports the concept that plaque stabilization might be achieved in a short-term period (median, 37-day therapy), although the relative contribution of statin LDL-C-lowering vs anti-inflammatory effect remained uninvestigated.¹⁸

The only previous prospective study¹² investigating the efficacy of a statin treatment (pravastatin) in modulating the plaque cell composition did not include a control group of patients using a nonstatin-based treatment. Other retrospective studies gave conflicting results about the effect of statins on cell composition of the carotid plaque.¹⁹ Comparison with our data are difficult because of differences in study design and the fact that we enrolled patients never treated with lipid-lowering medication. In our study, sitosterol plus cholestyramine and AT-10 induced a significant decrease in TC and LDL-C after a 3-month treatment, although the magnitude of such a change was greater with AT-10. Atorvastatin treatment was accompanied by lower macrophage content in carotid plaques (Figure 2A). Adjustment for the on-therapy LDL-C levels (Figure 2B) and for LDL-C changes with treatment (Supplemental Figure I) blunted the differences on plaque macrophage concentration among groups, although a trend was still observed. Of course, some of the residual effect on macrophages might be accounted for by changes in high-density lipoprotein cholesterol with treatment or other lipid parameters. To further define the lipid-dependent vs a nonlipid-dependent (pleiotropic) effect on plaque cell composition, a larger cohort of patients might be required, highlighting a potential limitation of our study. However, a recruitment of a larger high-risk and lipid-lowering naive population was limited by the current standard of treatment.

In the past few years, several *in vitro* and animal studies hypothesized a so-called pleiotropic effect of statins.⁷ Main nonlipid-related beneficial properties of statins include: (1) protective effect on endothelial function; (2) antithrombotic actions; and (3) anti-inflammatory effects. These additional

effects have been related to the blocking of HMG-CoA inhibitors on the mevalonate cascade that leads to reduced production of isoprenoids and inhibition of the Rho/Rho kinases pathway.⁷ This common mechanism upstream of the LDL-C-lowering and the pleiotropic effects of statin therapy are supported by the meta-analysis of Kinley¹¹ that clearly highlights that most of the anti-inflammatory effects of LDL-lowering therapies are related to the magnitude of change in LDL-C. Macrophage recruitment inside the atherosclerotic plaque represents a crucial event for atherosclerosis initiation, progression, and complication. Our finding of a decreased macrophage content within atherosclerotic lesions is in agreement with previous studies on animal models and humans.^{20–22} In our series we did not observe a significant change in total lymphocyte population content of the plaque. This finding may imply that short-term lipid-lowering does not result in modulation of the adaptive immune response, whereas some interference occurs in terms of innate immunity activation. We can speculate that the reduced macrophage accumulation could be followed-up during a longer period of treatment by a similar reduction in the lymphocyte population size, as suggested by the trend displayed in Figure 2A.

Previous clinical studies demonstrated that treatment with statin can lower the circulating level of inflammatory molecules, even in during short-term period.^{9,23} In our study we could not observe a significant effect of the treatments in reducing the level of several inflammatory markers, including high-sensitivity C-reactive protein. The lack of effect can be explained by the relatively low number of patients involved in our study compared to the high number of patients recruited in other clinical studies, which were not specifically designed to assess plaque cellularity. Nevertheless, based on our data, we could speculate that the reduced accumulation of macrophages observed in the lesions of patients treated with statins could anticipate the impact on systemic inflammation.

Conclusion

In conclusion, cellular plaque composition after short-term lipid-lowering therapy is significantly modulated by the degree of LDL-C-lowering. A contribution of LDL-independent, anti-inflammatory mechanisms on plaque stability is only suggested by our study. These data strongly support the current guidelines based on progressively lower LDL-C targets, depending on the cardiovascular risk of individual patients.

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Disclosures

None.

References

1. Grundy SM, Cleeman JJ, Merz CN, Brewer HB Jr, Clark LT, Hunnigake DB, Pasternak RC, Smith SC Jr, Stone NJ. A summary of implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Arterioscler Thromb Vasc Biol*. 2004;24:1329–1330.
2. LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, Gotto AM, Greten H, Kastelein JJ, Shepherd J, Wenger NK. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med*. 2005;352:1425–1435.
3. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207.
4. Schwartz GG, Olsson AG, Ezekowitz MD, Ganz P, Oliver MF, Waters D, Zeiher A, Chaitman BR, Leslie S, Stern T. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. *JAMA*. 2001;285:1711–1718.
5. Patti G, Pasceri V, Colonna G, Miglionico M, Fischetti D, Sardella G, Montinaro A, Di Sciascio G. Atorvastatin pretreatment improves outcomes in patients with acute coronary syndromes undergoing early percutaneous coronary intervention: results of the ARMYDA-ACS randomized trial. *J Am Coll Cardiol*. 2007;49:1272–1278.
6. Amarenco P, Bogousslavsky J, Callahan A III, Goldstein LB, Hennerici M, Rudolph AE, Silleen H, Simunovic L, Szarek M, Welch KM, Zivin JA. High-dose atorvastatin after stroke or transient ischemic attack. *N Engl J Med*. 2006;355:549–559.
7. Ray KK, Cannon CP. The potential relevance of the multiple lipid-independent (pleiotropic) effects of statins in the management of acute coronary syndromes. *J Am Coll Cardiol*. 2005;46:1425–1433.
8. Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *JAMA*. 2001;286:64–70.
9. Kinlay S, Schwartz GG, Olsson AG, Rifai N, Leslie SJ, Sasiela WJ, Szarek M, Libby P, Ganz P. High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study. *Circulation*. 2003;108:1560–1566.
10. McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakov O, Ford I, Capell HA, Sattar N. Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. *Lancet*. 2004;363:2015–2021.
11. Kinlay S. Low-density lipoprotein-dependent and -independent effects of cholesterol-lowering therapies on C-reactive protein: a meta-analysis. *J Am Coll Cardiol*. 2007;49:2003–2009.
12. Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation*. 2001;103:926–933.
13. Cannon CP, Steinberg BA, Murphy SA, Mega JL, Braunwald E. Meta-analysis of cardiovascular outcomes trials comparing intensive vs moderate statin therapy. *J Am Coll Cardiol*. 2006;48:438–445.
14. Barnett HJ, Taylor DW, Eliasziw M, Fox AJ, Ferguson GG, Haynes RB, Rankin RN, Clagett GP, Hachinski VC, Sackett DL, Thorpe KE, Meldrum HE, Spence JD. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. North Am Symptomatic Carotid Endarterectomy Trial Collaborators. *N Engl J Med*. 1998;339:1415–1425.
15. Pualetto P, Puato M, Faggin E, Santipolo N, Pagliara V, Zoleo M, Deriu GP, Grego F, Plebani M, Sartore S, Bon GB, Heymes C, Samuel JL, Pessina AC. Specific cellular features of atheroma associated with development of neointima after carotid endarterectomy: the carotid atherosclerosis and restenosis study. *Circulation*. 2000;102:771–778.
16. Sartore S, Chiavegato A, Franch R, Faggin E, Pualetto P. Myosin gene expression and cell phenotypes in vascular smooth muscle during development, in experimental models, and in vascular disease. *Arterioscler Thromb Vasc Biol*. 1997;17:1210–1215.
17. Puato M, Faggin E, Rattazzi M, Paterni M, Kozakova M, Palombo C, Pualetto P. In vivo noninvasive identification of cell composition of intimal lesions: a combined approach with ultrasonography and immunocytochemistry. *J Vasc Surg*. 2003;38:1390–1395.
18. Schouten O, Boersma E, Hoeks SE, Benner R, van Urk H, van Sambeek MR, Verhagen HJ, Khan NA, Dunkelgrun M, Bax JJ, Poldermans D. Fluvastatin and perioperative events in patients undergoing vascular surgery. *N Engl J Med*. 2009;361:980–989.
19. Verhoeven BA, Moll FL, Koekkoek JA, van der Wal AC, de Kleijn DP, de Vries JP, Verheijen JH, Velema E, Busser E, Schoneveld A, Virmani R, Pasterkamp G. Statin treatment is not associated with consistent alterations in inflammatory status of carotid atherosclerotic plaques: a retrospective study in 378 patients undergoing carotid endarterectomy. *Stroke*. 2006;37:2054–2060.
20. Sukhova GK, Williams JK, Libby P. Statins reduce inflammation in atheroma of nonhuman primates independent of effects on serum cholesterol. *Arterioscler Thromb Vasc Biol*. 2002;22:1452–1458.
21. Martin-Ventura JL, Blanco-Colio LM, Gomez-Hernandez A, Munoz-Garcia B, Vega M, Serrano J, Ortega L, Hernandez G, Tunon J, Egido J. Intensive treatment with atorvastatin reduces inflammation in mononuclear cells and human atherosclerotic lesions in one month. *Stroke*. 2005;36:1796–1800.
22. Cuccurullo C, Iezzi A, Fazio ML, De Cesare D, Di Francesco A, Muraro R, Bei R, Uccino S, Spigonardo F, Chiarelli F, Schmidt AM, Cuccurullo F, Mezzetti A, Cipollone F. Suppression of RAGE as a basis of simvastatin-dependent plaque stabilization in type 2 diabetes. *Arterioscler Thromb Vasc Biol*. 2006;26:2716–2723.
23. Ridker PM, Rifai N, Lowenthal SP. Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia. *Circulation*. 2001;103:1191–1193.