Atorvastatin Reduces Macrophage Accumulation in Atherosclerotic Plaques
A Comparison of a Nonstatin-Based Regimen in Patients Undergoing Carotid Endarterectomy

Massimo Puato, MD; Elisabetta Faggini, PhD; Marcello Rattazzi, MD, PhD; Alberto Zambon, MD; Francesco Cipollone, MD; Franco Grego, MD; Lorenzo Ganassin, MD; Mario Plebani, MD; Andrea Mezzetti, MD; Paolo Pauletto, MD

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. However, a short-term benefit has been documented for patients treated with statins in acute coronary syndromes and other clinical settings. These observations strengthened the hypothesis of additional so-called pleiotropic actions of statins. For instance, several clinical studies demonstrated an anti-inflammatory effect of treatment with statins (such as C-reactive protein-lowering) 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 contribute to the reduction of inflammatory biomarkers in statin-treated patients. In addition, we do not known 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 al 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.
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 ≥70% (NASCET criteria), 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 ClinicalTrial.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-1β, 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-1β, IL-10, and tumor necrosis factor-α were determined by chemiluminescent immunometric assay on the Immulite 1000 analyzer (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. 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 antimonocyte-macrophage (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. 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. According to a method previously validated 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±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, glycemia, 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
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)).

### Table. Baseline Demographic Data and Lipids Profile Among the Study

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

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 $\chi^2$ for categorical variables and analysis of variance for continuous variables.

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×.
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. 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, we observed an increase in the number of SM cells in the plaque, at least in patients treated with the

![Figure 2](http://stroke.ahajournals.org/)

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.
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.

The only previous prospective study 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. 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 cholesteryamine 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. 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. This common mechanism upstream of the LDL-C–lowering and the pleiotropic effects of statin therapy are supported by the meta-analysis of Kinley 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.

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
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Original Contributions

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 Objectives: To compare the effects of high and low dose atorvastatin with a non-steroidal anti-inflammatory drug on macrophage accumulation in atherosclerotic plaques.

Methods: A total of 60 patients with hypercholesterolemia (total cholesterol 5.83–7.64 mmol/L) were randomized to three groups (n=20): atorvastatin 10 mg/day (AT-10), atorvastatin 80 mg/day (AT-80), and simvastatin 80 mg/day plus fenofibrate (SM-VF). The arterial wall was harvested at arterectomy and analyzed for macrophage, lymphocyte, and smooth muscle cell content.

Results: All groups had significant reductions in total cholesterol and low-density lipoprotein cholesterol (LDL-C), but the non-steroidal group showed less reduction. No significant changes were observed in inflammatory markers, including high-sensitivity C-reactive protein. Compared to the non-steroidal group, AT-10 and AT-80 both reduced macrophage numbers, with AT-80 being more effective. After adjusting for LDL-C changes, these differences were no longer statistically significant. No differences were observed in lymphocyte numbers, but smooth muscle cell numbers were higher in the AT-80 group. LDL-C changes were negatively correlated with macrophage numbers.

Conclusion: High-dose atorvastatin treatment reduces macrophage accumulation in atherosclerotic plaques more than a non-steroidal regimen, and this effect is dose-dependent.

Keywords: Atherosclerosis, Carotid Artery, Lipid, Lipoprotein, Macrophage, Statin


基于以上原因，我们针对三个月内使用不同降脂策略(非他汀治疗、低剂量他汀治疗、高剂量他汀治疗)以及他汀治疗是否影响颈动脉斑块的细胞构成进行了调查，并特别注意区别LDL-C降低对斑块细胞构成的影响与他汀不依赖于脂质的作用对斑块内巨噬细胞和平滑肌细胞的影响。

受试者和方法

研究设计

我们从三个研究中心共纳入60例符合颈动脉内膜切除术适应症的患者，所纳入患者均是从未接受降脂治疗的高胆固醇血症患者(总胆固醇[TC]范围，5.83-7.64 mmol/L)，且均伴有症状性颈动脉狭窄[14]。所有患者的入组时间均发生在临床事件后20-30天内，随机分到三个治疗组：阿
托伐他汀 10 mg/天组 (AT-10 组) 、阿托伐他汀 80 mg/天组 (AT-80 组) 、消胆胺 (如奎仑，百时美施贵宝) 8 g/天加谷固醇 (联合利华) 2.5 g/天组 (C-S 组)。每个治疗组均纳入 20 例患者，在内膜切除术前用药 3 个月。患者开始药物治疗后 12 周 ±2 天时接受颈动脉内膜切除术。由于该人群心血管风险高，考虑到伦理问题，本研究未设置安慰剂对照组。

该试验通过了当地伦理委员会的审批并在 ClinicalTrial.org (CT Identifier: NCT01053065) 注册。所有患者签署了知情同意书。

血样分析

研究开始时和手术时抽取患者血样，测定血脂 (TC、LDL-C、高密度脂蛋白胆固醇、甘油三酯)，炎症标记物 (高敏 C 反应蛋白、IL-6、IL-8、IL-10、IL-1β、RANTES、单核细胞趋化蛋白-1、肿瘤坏死因子-α、sCD40L) 和粘附分子 (可溶性 P 选择素、可溶性血管性细胞粘附分子-1[sVCAM-1])。使用 1000 型全自动化学发光免疫分析仪 (IMMULITE; Siemens Diagnostics) 通过化学发光免疫技术测定血清 IL-6、IL-8、IL-10、IL-1β、肿瘤坏死因子-α水平。使用酶联免疫吸附法 (BioSource International) 测定可溶性 P 选择素、sCD40L、单核细胞趋化蛋白-1、sVCAM-1、RANTES 水平。使用浊度测定法定量测定血清 C 反应蛋白、C3 和 C4 水平。

测定颈动脉斑块内细胞构成和脂质成分

术后立即使用液氮对内膜切除标本快速冷冻，并嵌入 OCT(Sakura)，然后储存在 -80 ℃。标本进行 8 μm 厚切片，随后使用先前报道的免疫组化技术处理 [15]，并用下列单克隆抗体测定病变内的细胞成分: SM-E7 抗平滑肌 (SM) 肌球蛋白重链抗体、HAM-56 抗单核巨噬细胞抗体 (Dako) 和 CD45RO 抗淋巴细胞抗体 (Dako)。SM-E7 与 SM 型肌球蛋白重链 (SM1 和 SM2) 特异性结合用以识别 SM 系列的所有细胞 [16]。除 CD45RO 抗体外，初级抗体均应用于新鲜未固定 8 μm 厚切片。用于间接免疫组化的对照剂是鼠非免疫性 IgG 抗体而不是单独使用的初级抗体和次级抗体。使用苏木精-伊红染色显示细胞核；使用标准苏丹黑染色方案显示斑块内脂质成分。

颈动脉切除标本的图像分析

使用 Qwin 数码相机 (莱卡) 对染色标本照片并用于图像分析 [17]。根据先前证实有效的方法 [15,17]，一个抗体测定中，对每个标本选取三张切片，每张切片选取三个标准显微镜视野，但排除外弹性膜下底层的切片和存在非典型染色区域的切片。各种抗体的阳性染色用其占总区域的百分比表示。通过计数邻近切片苏木精染色阳性细胞核来判断斑块的细胞完整性。通过斑块的细胞完整性来校正每一抗体的染色区域。

通过苏丹黑染色阳性区域评估斑块内脂质成分，并用其占总斑块区域百分比表示，由两个调查者 (盲法) 独立分析。统计分析

使用均数 ± 标准差表示连续性变量。使用方差分析和邦弗朗尼 (Bonferroni) 更正比较受试者。校正细胞结构完整性后使用协方差分析不同细胞类型的阳性面积。P<0.05 时认为有统计学意义。使用 SPSS 软件包进行统计分析。

结果

患者基线特征和治疗对脂质成分的效果

三组患者在颈动脉狭窄程度、年龄、性别、血压、血糖和血脂等方面没有差异 (表)。所有患者均使用抗血小板药 (如阿司匹林或噻氯匹定)。三种治疗方案的患者 TC、LDL-C 和非高密度脂蛋白胆固醇均明显降低 (表)。AT-10 组和 AT-80 组患者 TC 和 LDL-C 变化没有差异，而 C-S 组患者 TC 和 LDL-C 降低幅度明显低于 AT-10 组 (P<0.0005) 和 AT-80 组 (P<0.0005) 患者。C-S 组患者非高密度脂蛋白胆固醇降低程度较小。研究结束时，三组患者血清高密度脂蛋白胆固醇和甘油三酯水平没有差异。

三组患者没有出现临床明显副反应或严重不良事件。

降脂治疗对血液中炎性标记物的影响

三组患者高敏 C 反应蛋白水平基线时 (AT-10, 4.72 ±3.90 mg/L; AT-80, 2.87 ±3.03 mg/L; C-S, 3.39 ±2.05 mg/L) 和研究结束时 (AT-10, 2.87 ±2.62 mg/L; AT-80, 2.21 ±2.52 mg/L; C-S, 2.73 ±4.47 mg/L) 没有差异。三种治疗方案均没有明显改变血液中各种促炎因子水平。其他炎性标记物如 RANTES 或补体水平 (C3-C4) 亦未受影响。

颈动脉斑块的细胞学和形态学特征

与 C-S 组患者相比，AT-10 组患者颈动脉内膜标志斑块内巨噬细胞聚集明显较低，而 AT-80 组则降低更明显 (图 1 和 2A)。动脉粥样硬化斑块内平滑肌细胞的变化趋势则与巨噬细胞相反。AT-10 组和 AT-80 组患者斑块中平滑肌细胞数量高于 C-S 组，AT-80 组与 C-S 组差距更明显 (图 1 和 2A)。考虑到三种治疗方案对 LDL-C 的影响显著不同，我们分别校正治疗后 LDL-C 水平和 LDL-C 变化水平进行统计分析。
行分析。校正治疗后 LDL-C 水平后，AT-80 组患者斑块内巨噬细胞聚集仍然明显低于 C-S 组 (图 2B)。校正 LDL-C 变化水平后，三组患者仍然观察到较低巨噬细胞聚集水平和更高的平滑肌细胞聚集水平，但没有统计学意义 ( 补充图 1，可在线获得http://stroke.ahajournals.org)。

三组患者斑块内淋巴细胞数量相似且没有受到治疗的影响。三组患者动脉粥样硬化斑块内脂质成分相似 (斑块区域 %: C-S, 35±16; AT-10, 37±25; AT-80, 28±19)。

通过直线回归分析，发现三组患者的 LDL-C 变化水平与动脉粥样斑块内巨噬细胞聚集成明显负相关 ($r=-0.456; P=0.007$; 图 3)，而 LDL-C 变化水平与斑块内平滑肌细胞数量呈正相关趋势，但没有统计学意义 (图 3)。

讨论

据我们所知，本研究第一次证实阿托伐他汀短期治疗优于非他汀降脂治疗，且这种效果是由 LDL-C 变化水平调节的。正如我们之前的预测，AT-80 组 TC 和 LDL-C 降低最明显，其伴随最相关的变化是斑块内细胞结构重塑。MIRACL 研究证实早期使用阿托伐他汀

### 表 基线人口统计学资料和研究过程中血脂谱

<table>
<thead>
<tr>
<th>变量</th>
<th>谷固醇 + 消胆胺 (n=20)</th>
<th>阿托伐他汀 10 mg (n=20)</th>
<th>阿托伐他汀 80 mg (n=20)</th>
<th>$P$ 值</th>
</tr>
</thead>
<tbody>
<tr>
<td>年龄, 岁</td>
<td>78.7 ± 5.3</td>
<td>78.4 ± 5.1</td>
<td>79.0 ± 5.0</td>
<td>NS (0.930)</td>
</tr>
<tr>
<td>男性</td>
<td>11</td>
<td>7</td>
<td>9</td>
<td>NS (0.522)</td>
</tr>
<tr>
<td>收缩压, mm Hg</td>
<td>142 ± 16</td>
<td>142 ± 15</td>
<td>146 ± 17</td>
<td>NS (0.609)</td>
</tr>
<tr>
<td>舒张压, mm Hg</td>
<td>81 ± 12</td>
<td>83 ± 12</td>
<td>79 ± 11</td>
<td>NS (0.581)</td>
</tr>
<tr>
<td>bs-CRP T1, mg/L</td>
<td>3.34 ± 2.00</td>
<td>3.59 ± 2.39</td>
<td>2.33 ± 1.68</td>
<td>NS (0.145)</td>
</tr>
<tr>
<td>总胆固醇 T1, mmol/L</td>
<td>6.81 ± 0.44</td>
<td>7.04 ± 0.26</td>
<td>6.94 ± 1.09</td>
<td>NS (0.667)</td>
</tr>
<tr>
<td>总胆固醇 T2, mmol/L</td>
<td>6.24 ± 0.73</td>
<td>7.52 ± 0.85</td>
<td>5.31 ± 0.78</td>
<td>0.002</td>
</tr>
<tr>
<td>LDL-C T1, mmol/L</td>
<td>-0.60 ± 0.47</td>
<td>-1.40 ± 0.49</td>
<td>-1.75 ± 0.60</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>LDL-C T2, mmol/L</td>
<td>-0.59 ± 0.34</td>
<td>-1.30 ± 0.65</td>
<td>-1.50 ± 0.75</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>HDL-C T1, mmol/L</td>
<td>1.24 ± 0.28</td>
<td>1.19 ± 0.26</td>
<td>1.48 ± 0.60</td>
<td>NS (0.071)</td>
</tr>
<tr>
<td>HDL-C T2, mmol/L</td>
<td>1.24 ± 0.18</td>
<td>1.17 ± 0.10</td>
<td>1.40 ± 0.49</td>
<td>NS (0.115)</td>
</tr>
<tr>
<td>δHDL-C, mmol/L</td>
<td>0.01 ± 0.14</td>
<td>0.20 ± 0.11</td>
<td>-0.10 ± 0.17</td>
<td>0.034</td>
</tr>
<tr>
<td>Non-HDL-C T1, mmol/L</td>
<td>5.57 ± 0.52</td>
<td>5.88 ± 0.80</td>
<td>5.46 ± 1.53</td>
<td>NS (0.458)</td>
</tr>
<tr>
<td>Non-HDL-C T2, mmol/L</td>
<td>5.00 ± 0.78</td>
<td>4.56 ± 0.85</td>
<td>3.91 ± 1.09</td>
<td>0.003</td>
</tr>
<tr>
<td>δNon-HDL-C, mmol/L</td>
<td>-0.62 ± 0.49</td>
<td>-1.42 ± 0.52</td>
<td>-1.55 ± 0.65</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>甘油三酯 T1, mmol/L</td>
<td>1.62 ± 0.88</td>
<td>1.49 ± 0.70</td>
<td>1.31 ± 0.53</td>
<td>NS (0.423)</td>
</tr>
<tr>
<td>甘油三酯 T2, mmol/L</td>
<td>1.33 ± 0.43</td>
<td>1.28 ± 0.24</td>
<td>1.31 ± 0.44</td>
<td>NS (0.904)</td>
</tr>
<tr>
<td>δ甘油三酯, mmol/L</td>
<td>-0.25 ± 0.55</td>
<td>-0.28 ± 0.70</td>
<td>-0.02 ± 0.23</td>
<td>NS (0.209)</td>
</tr>
</tbody>
</table>

连续性变量使用均数 ± 标准差表示。
HDL-C 代表高密度脂蛋白胆固醇; bs-CRP 代表高敏 C 反应蛋白; NS 代表没有统计学意义; T1 代表基线; T2 代表随访; δ 代表差值 (T2 值 - T1 值)。
分类变量使用 $\chi^2$ 检验进行统计分析，连续性变量使用方差分析进行统计分析。

### 图 1 治疗 3 个月后颈动脉斑块的细胞特征

三个治疗组颈动脉斑块内巨噬细胞 (HAM-56)、平滑肌细胞 (SM-E7) 和淋巴细胞 (CD-45RO) 免疫组化染色的代表性图片; 点线代表斑块 (pl) 和内膜的分界线 (m); 放大倍数 100×。
他汀 80 mg/ 天进行四个月的强化降脂治疗能降低急性冠脉综合症患者再发缺血性事件的风险 [4]。本研究发现使用阿托伐他汀 80 mg/ 天治疗可使斑块内巨噬细胞降低最多, 提示阿托伐他汀调节 LDL-C 的剂量依赖性, 在病理生理水平上对 MIRACL 研究结论 (他汀类治疗有益) 提供了有力的证据。目前已知巨噬细胞活化产物如基质金属蛋白酶、活性氧簇等可通过增加斑块破裂风险而损害纤维帽的完整性。因此, 使用他汀降低斑块内巨噬细胞数量可能是促进斑块完整性的因素。目前仅有少数人体研究显示, 他汀治疗后斑块内平滑肌细胞数量增加 [12], 本研究将其与之比较, 探索至少在使用最高他汀剂量组即 AT-80 组斑块内平滑肌细胞数量是增加的。该发现进一步提示更稳定的斑块是使用他汀治疗产生的结果, 即使是短期内使用他汀治疗。最近一项关于血管手术患者使用他汀治疗的研究支持这一理念; 斑块稳定性可在短期治疗时间内 (中位数, 37 天) 获得, 尽管这一效果是源于他汀治疗后 LDL-C 降低还是源于他汀抗炎效果有待进一步研究 [18]。

既往有一项前瞻性研究 [12] 评价了他汀治疗调整斑块内细胞构成的疗效, 但该研究没有纳入使用非他汀降脂药物的对照组。其他关于他汀调整斑块内细胞构成的回顾性研究的结论互相矛盾 [19]。很难将这些研究的结论与我们进行比较, 因各自的研究设计不同且我们纳入的是从未接受过降脂药物治疗的患者。本研究中谷固醇联合消胆胺与 AT-10 治疗 3 个月后均使 TC 和 LDL-C 明显降低, AT-10 组的降低幅度更大。阿托伐他汀治疗使斑块内巨噬细胞数量降低 (图 2)。校正治疗后 LDL-C 水平后分析细胞成分; 校正治疗后 LDL-C 水平后, AT-80 组患者巨噬细胞数量仍然明显低于 C-S 组患者。
脂质依赖效应，还需要募集数量更多的患者，这也是本研究的一个局限。但是，收集更多风险且未使用降脂治疗的患者会受到目前治疗规范的限制。在过去几年中，几个体外实验和动物实验提出了他汀治疗的多种效应假说[7]。他汀治疗主要的非脂质依赖改变的有利作用包括：(1) 保护内皮功能；(2) 抗栓作用；(3) 抗炎作用。这些附加效果与阻断HMG-CoA抑制剂所不同，从而使HMG-CoA抑制剂不能用于羟基酸级联反应，降低异戊二烯的生成并抑制Rho/Rho激酶途径[8]，这是他汀治疗降低LDL-C效应及其多种效应的共同上级机制。Kinley的Meta分析[11]支持这一机制，并明确强调绝大多数降LDL-C治疗的抗炎效应与LDL-C改变幅度相关。动脉粥样硬化斑块内巨噬细胞聚集是导致动脉粥样硬化启动、进展和发生并发症至关重要的因素。我们发现动脉粥样硬化斑块内巨噬细胞数量降低与先前的动物模型和人体试验一致[26-22]。本研究未发现斑块内淋巴细胞系有明显改变，这可能暗示短期降脂治疗不会导致适应性炎症反应发生变化，而固有免疫反应可能产生一些冲突。正如图2A所示的趋势，我们推测如果随访更长时间可能会发现斑块内淋巴细胞数量出现与巨噬细胞类似的降低趋势。目前，他汀治疗对几种炎性标记物(包括高敏C反应急蛋白)明显的降低作用可能的解释是，与其他纳入更多患者的临床研究(不是专门设计以评估斑块细胞的完整性的研究)相比，我们纳入的患者数量相对较少。尽管如此，根据我们的数据，可以推测他汀治疗患者斑块内巨噬细胞数量的降低可影响系统炎症。结论

本研究结果提示短期降LDL-C治疗后细胞斑块构成变化的调节与LDL-C降低程度显著相关。降脂治疗独立于LDL-C改变的抗炎机制影响斑块稳定性。本研究数据强烈支持近期指南关于应基于个体心血管病风险制定不同的降低LDL-C目标水平的推荐。

参考文献