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;41:00-00.)

Key Words: atherosclerosis ■ carotid artery ■ lipids ■ lipoprotein ■ macrophages ■ statins
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 lipoid-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),

*subjects 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 (Questen, 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 (Immulumite; 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 non-high-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 non-high-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>
<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>
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<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>
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<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>
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<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>
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<td>4.56±0.85</td>
<td>3.91±1.09</td>
<td>0.003</td>
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<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>
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<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>−0.28±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 χ² 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×.

Table. Baseline Demographic Data and Lipids Profile Among the Study
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
hypothesized a so-called pleiotropic effect of statins. Main population was limited by the current standard of treatment. Recruitment of a larger high-risk and lipid-lowering naive highlighting a potential limitation of our study. However, a position, a larger cohort of patients might be required, nonlipid-dependent (pleiotropic) effect on 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, siosterol plus cholesterol 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 LDL-C levels (Figure 2B) and for LDL-C changes with treatment (Supplemental Figure I) blunted the differences on LDL-C levels. Of course, some of the residual change in LDL-C may be accounted for changes in 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 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.

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 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. 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 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.

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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Leopoldo Pagliani, MD; Carmen Tirritolo, MD; Florian Amor, MD; and Marco Zanardo, MD, are gratefully acknowledged for their contribution to enrollment of patients and specimens collection.

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Disclosures

None.

References


Cellular features of carotid plaques

- Sitosterol + Cholestyramine
- Atorvastatin 10 mg/d
- Atorvastatin 80 mg/d

Data adjusted for LDL-cholesterol changes

(ANOVA n.s. = 0.147) (ANOVA n.s. = 0.117) (ANOVA n.s. = 0.773)

Figure 1. Analysis of cellular content of carotid plaques adjusted for changes in LDL-C levels. Lower macrophage content and higher smooth muscle cells concentration was still observed after adjustment for changes in LDL-C in the three groups, although differences were no longer significant.
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 doses of atorvastatin and a nonstatin-based regimen (cholesterol and cholestyramine) on the cellular composition of carotid plaques.

Methods: A total of 60 patients with symptomatic carotid stenosis (≥70%) were randomly assigned to receive atorvastatin 10 mg/day (AT-10), atorvastatin 80 mg/day (AT-80), cholesterol 2.5 g/day, and cholestyramine 8 g/day. The cellular composition of the endarterectomy samples was analyzed.

Results: All treatment groups showed a reduction in total cholesterol and LDL-C levels, but the cholestyramine and cholesterol group showed a lesser reduction in LDL-C levels. No significant changes were observed in inflammatory markers (hs-CRP). Compared to the cholesterol and cholestyramine group, the AT-10 group had a significantly lower number of macrophages, and the AT-80 group had a more pronounced decrease. After adjusting for LDL-C changes, these changes were not statistically significant. The number of lymphocytes within the plaques did not differ among the groups, while the number of smooth muscle cells was higher in the AT-80 group. A negative correlation was observed between LDL-C changes and the number of macrophages within the plaques.

Conclusions: High-dose atorvastatin treatment was superior to a nonstatin-based regimen in reducing macrophage accumulation in carotid plaques, and the effect depended on the degree of LDL-C reduction.

Keywords: Atherosclerosis, Carotid, Lipids, Lipoprotein, Macrophage, Statin

Original Contributions

颈动脉内膜切除术患者阿托伐他汀与非他汀类降脂药物降低动脉粥样硬化斑块内巨噬细胞聚集作用的比较

从颈动脉内膜切除术获取的患者样本中，高剂量阿托伐他汀与非他汀类降脂药物降低动脉粥样硬化斑块内巨噬细胞聚集作用的比较，表明高剂量阿托伐他汀在降低动脉粥样硬化斑块内巨噬细胞聚集方面优于非他汀类降脂药物。此发现支持了他汀类药物在预防心血管事件方面的作用，特别是通过降低LDL-C水平来影响斑块内巨噬细胞的聚集。
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（如奎传, 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 E 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 E 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冰冻, 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) 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的组织切片 (8 μm 厚)。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。

内膜切除标本切片的图像分析

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], 每一个抗体测定中, 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

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 变化水平没有差异, 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 (IL-6、IL-8 和肿瘤坏死因子-α)。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本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 组患者斑块切片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
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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 I，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，本研究第一次证实降低动脉粥样硬化斑块内巨噬细胞数量方面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

<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>性别</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>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>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>6.51 ± 0.78</td>
<td>0.002</td>
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<tr>
<td>Δ 总胆固醇, 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>1.40 ± 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>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 ± 0.23</td>
<td>NS (0.209)</td>
</tr>
</tbody>
</table>

近似性变量使用均数 ± 标准差表示。

HDL-C 代表高密度脂蛋白胆固醇; hs-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)。

连续性变量使用 t 检验进行统计分析，分类变量使用 χ² 检验进行统计分析。
The 80 mg/day atorvastatin for four months of intensive lipid lowering therapy reduces the risk of recurrent ischemic events in patients with acute coronary syndrome [4]. This study found that atorvastatin 80 mg/day treatment can reduce the number of macrophages in the plaque most, suggesting that atorvastatin regulates LDL-C in a dose-dependent manner, providing strong evidence for the MIRACL study conclusion (that statin treatment is beneficial) [5]. It is now known that macrophage-derived products such as matrix metalloproteinases, reactive oxygen species, etc. can increase plaque rupture risk by damaging the fibrous cap. Therefore, using statins to reduce the number of macrophages in the plaque may be an important factor in promoting plaque stability. Prior to this study, only one human study demonstrated that statin treatment increases the number of smooth muscle cells [12]. This study is consistent with this finding, finding that at least in the highest atorvastatin dose group (AT-80), the number of smooth muscle cells in the plaque is increased. This finding further suggests that a more stable plaque expression is a result of statin treatment, even in the short term. A recent study on vascular surgery patients who received statins supports this idea: plaque stability can be achieved in the short treatment period (median, 37 days) despite the fact that this effect is derived from lowering LDL-C post treatment rather than the anti-inflammatory effect of statins [18].

In a previous prospective study [12], the effects of statins on adjusting plaque cell composition were evaluated, but this study did not include a non-statin lipid-lowering drug control group. Other retrospective studies on statins adjusting plaque cell composition had contradictory conclusions [19]. It is difficult to compare the results of these studies with our findings because of different study designs and our inclusion of patients who had never received lipid-lowering drugs. In this study, the combination ofsimvastatin and cholestyramine reduced TC and LDL-C levels for 3 months, with a greater reduction in the AT-80 group. Atorvastatin treatment reduced the number of macrophages in the plaque (Figure 2). After adjustment for the LDL-C level post-treatment, the number of macrophages was still significantly lower in the AT-80 group compared to the C-S group (Bonferroni p=0.031). The number of smooth muscle cells in the plaque was not significantly different among the groups (Bonferroni n.s.=0.621), while the number of lymphocytes was significantly increased in the AT-80 group (Bonferroni p=0.003). Cell structure was expressed as the percentage of the area stained with specific antibodies containing normal cell nuclei in the plaque region. After adjusting for LDL-C levels post-treatment, the number of macrophages in the AT-80 group was significantly lower compared to the C-S group (Bonferroni p=0.013). The number of smooth muscle cells and lymphocytes was not significantly different among the groups (Bonferroni n.s.=0.560). The number of macrophages was significantly lower in the AT-80 group compared to the C-S group after adjustment for LDL-C levels and follow-up data (Bonferroni p=0.031).
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的样本将会对治疗规范的限制。

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。例如，HMG-CoA还原酶抑制剂可以降低巨噬细胞数量，降低亚硝酸的磷脂质降解，影响巨噬细胞的增殖和凋亡。此外，他汀治疗可以降低LDL-C水平，降低巨噬细胞数量。

因此，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能影响炎症。

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。本研究结果强烈支持近期指南中关于基于个体心血管疾病风险制定不同的降低LDL-C目标水平的推荐。

参考文献

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