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

Massimo Puato, MD; Elisabetta Faggin, 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.1–4 However, a short-term benefit has been documented for patients treated with statins in acute coronary syndromes4,5 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 contribute 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
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),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 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 immunoassay 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.14 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 antibodies. The SM-E7 reacts with SM-type myosin heavy chains (both SM1 and SM2) exclusively and recognizes all cells in the SM lineage.15 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 validated12,17 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±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 effect 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>1.40±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>
</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>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>-0.02±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×.
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 \((r=−0.456; \ P=0.007; \text{Figure 3})\). 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 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.
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 lipid parameters. To further define the lipid-dependent vs a effect on macrophages might be accounted for by changes in plaque macrophage concentration among groups, although a magnitude of such a change was greater with AT-10. Atorvastatin and LDL-C after a 3-month treatment, although the magni- 

tude of 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 athero-sclerotic 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 mole- 
cules, 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 accumulation of macrophages observed in the lesions of patients treated with statins could anticipate the impact on systemic inflammation.

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 sta-

bility 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.

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

Sources of Funding
The Biomedical Foundation for Cardiovascular Research and Gene Therapy of Padova, Italy (a non-profit institution), has provided generous financial support to this study. The authors also acknowledge Pfizer for partially supporting this study by an unrestricted educational grant.
Disclosures

None.

References


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*Stroke*. 2010;41:1163-1168; originally published online April 22, 2010; doi: 10.1161/STROKEAHA.110.580811

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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http://stroke.ahajournals.org/content/41/6/1163

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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

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

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背景和目的: 比较高、低剂量阿托伐他汀和非他汀治疗(谷固醇加消胆胺)对颈动脉斑块细胞构成的影响。

方法: 共纳入60例符合颈动脉内膜切除术适应症的高胆固醇血症患者(总胆固醇5.83-7.64 mmol/L)。患者于术前3个月随机分为三组(n=20): 阿托伐他汀10 mg/天(AT-10)、阿托伐他汀80 mg/天(AT-80)、消胆胺8 g/天加谷固醇2.5 g/天。分析各组内膜切除标本的细胞构成。

结果: 三组患者的血清总胆固醇和低密度脂蛋白胆固醇(LDL-C)水平均明显降低，但消胆胺加谷固醇组患者总胆固醇和LDL-C降低程度较少。三种治疗方案均没有显著改变炎症标记物水平(包括高敏C反应蛋白)，与消胆胺加谷固醇组患者比较，AT-80组患者斑块内巨噬细胞数量未见差异，而平滑肌细胞数量在AT-80组更高。三组患者LDL-C变化量与斑块内巨噬细胞数量呈负相关。

结论: 短期高剂量阿托伐他汀治疗在降低动脉粥样硬化斑块内巨噬细胞数量方面优于非他汀降脂疗法，其降巨噬细胞的效果取决于LDL-C降低程度。

关键词: 动脉粥样硬化，颈动脉，脂质，脂蛋白，巨噬细胞，他汀

(Stroke. 2010;41:1163-1168. 王德任 译 刘鸣 张世洪 校)
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 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) 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 (Dako) 抗体。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], 每一个抗体测定时,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异性染色区域的切片。各

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目高于 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

### 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>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>5.72 ± 0.85</td>
<td>5.31 ± 0.78</td>
<td>0.002</td>
</tr>
<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>
</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>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>-0.02 ± 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; 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)。

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 个月后动脉粥样斑块的细胞特征。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，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重要因素。此前仅有一项人体研究显示，阿托伐他汀治疗后斑块内平滑肌细胞数量增加[12]，本研究与其一致，发现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表达是使用他汀治疗产生结果的，尤其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果与我们进行比较，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（图2B）和LDL-C变化水平（补充图I）后，组间斑块内巨噬细胞数量的差异减弱，但仍然观察到同向的趋势。当然，对巨噬细胞的一些残留效应可由治疗后高密度脂蛋白胆固醇或其他脂类的水平变化来解释。要想进一步确定对斑块内细胞构成的影响是脂质依赖还是非
脂质依赖效应，还需要募集更多患者，这里没有明显的降低作用。可能的解释是，与其他纳入更多患者的研究不同，他汀治疗可以降低循环中炎性标记物，尤其是脂质依赖效应，还需要募集更多患者，这也限制了他汀治疗的多种效应的共同上级机制。

脂质依赖效应，还需要募集更多患者，这是一方面的局限。但是，收集更多高风险且未使用降脂治疗的患者会受到限制的影响。在过去几年，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，如降脂和抗炎作用；(2) 抗栓作用；(3) 抗炎作用。这些附加效应与阻断 HMG-CoA 还原酶抑制剂相关，从而使 HMG-CoA 还原酶抑制剂不能作用于甲羟戊酸级联反应，降低异戊二烯的生成并抑制 Rhino。Rho 蛋激酶途径[3]，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[4]支持这一机制，并强调调降大量 LDL-C 治疗措施的抗炎效果与 LDL-C 改变幅度相关。动脉粥样硬化斑块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巨噬细胞数量降低与先前的动物模型和人体试验一致[20-22]。本研究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免疫反应发生调节，而固有免疫反应可能会产生一些冲突。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变化。对于两种类型的巨噬细胞，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。}

图 3 三组患者 LDL-C 变化量与动脉粥样斑块细胞特征的相关性。A. 表示随访后基线值计算得到 LDL-C 变化量。B. 细胞结构用含 C- 反应蛋白的二硫代双丙氨酸标记。C. 表示 LDL-C 水平降低巨噬细胞数量逐渐降低。右图显示随着 LDL-C 水平降低平滑肌细胞数量增加。

脂质依赖效应，还需要募集更多患者，这是一方面的局限。但是，收集更多高风险且未使用降脂治疗的患者会受到限制的影响。在过去几年，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，如降脂和抗炎作用；(2) 抗栓作用；(3) 抗炎作用。这些附加效应与阻断 HMG-CoA 还原酶抑制剂相关，从而使 HMG-CoA 还原酶抑制剂不能作用于甲羟戊酸级联反应，降低异戊二烯的生成并抑制 Rhino。Rho 蛋激酶途径[3]，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[4]支持这一机制，并强调调降大量 LDL-C 治疗措施的抗炎效果与 LDL-C 改变幅度相关。动脉粥样硬化斑块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巨噬细胞数量降低与先前的动物模型和人体试验一致[20-22]。本研究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免疫反应发生调节，而固有免疫反应可能会产生一些冲突。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变化。对于两种类型的巨噬细胞，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。