Pravastatin Treatment Increases Collagen Content and Decreases Lipid Content, Inflammation, Metalloproteinases, and Cell Death in Human Carotid Plaques

Implications for Plaque Stabilization

Milita Crisby, MD; Gunilla Nordin-Fredriksson, MD; Prediman K. Shah, MD; Juliana Yano, BS; Jenny Zhu, BS; Jan Nilsson, MD, PhD

Background—The clinical benefits of lipid lowering with statins are attributed to changes in plaque composition leading to lesion stability, but supporting clinical data from human studies are lacking. Therefore, we investigated the effect of 3 months of pravastatin treatment on composition of human carotid plaques removed during carotid endarterectomy.

Methods and Results—Consecutive patients with symptomatic carotid artery stenosis received 40 mg/d pravastatin (n=11) or no lipid-lowering therapy (n=13; control subjects) for 3 months before scheduled carotid endarterectomy. Carotid plaque composition was assessed with special stains and immunocytochemistry with quantitative image analysis. Plaques from the pravastatin group had less lipid by oil red O staining (8.2±8.4% versus 23.9±21.1% of the plaque area, P<0.05), less oxidized LDL immunoreactivity (13.3±3.6% versus 22.0±6.5%, P<0.001), fewer macrophages (15.0±10.2% versus 25.3±12.5%, P<0.05), fewer T cells (11.2±9.3% versus 24.3±13.4%, P<0.05), less matrix metalloproteinase 2 (MMP-2) immunoreactivity (3.6±3.9% versus 8.4±5.3%, P<0.05), greater tissue inhibitor of metalloproteinase 1 (TIMP-1) immunoreactivity (9.0±6.2% versus 3.1±3.9%, P<0.05), and a higher collagen content by Sirius red staining (12.4±3.1% versus 7.5±3.5%, P<0.005). Cell death by TUNEL staining was reduced in the pravastatin group (17.7±7.8% versus 32.0±12.6%, P<0.05).

Conclusions—Pravastatin decreased lipids, lipid oxidation, inflammation, MMP-2, and cell death and increased TIMP-1 and collagen content in human carotid plaques, confirming its plaque-stabilizing effect in humans. (Circulation. 2001;103:926-933.)

Key Words: pravastatin ■ plaque ■ inflammation

Several trials have demonstrated reduced cardiovascular events and mortality with lipid-lowering therapy.1–5 An- giographic studies show less progression, more regression, and less new lesion development with lipid-lowering ther- apy.6–9 Greater clinical event reduction, however, compared with only modest changes in stenosis severity, suggests that lipid lowering may produce clinical benefit largely by “sta- bilizing” plaques against disruption.6–9 This concept of plaque stabilization is predicated on the principle that a reduction in lipid content and inflammatory activity, 2 of the major determinants of plaque instability, would reduce the risk of plaque disruption, thereby preventing adverse clinical events.9–12 Although animal studies have supported this hypothesis, data in humans are lacking.13–16 Therefore, in this study, we determined the effect of 3 months of lipid-lowering therapy with pravastatin on carotid plaque composition in patients undergoing carotid endarterectomy (CEA) for symptomatic severe internal carotid artery stenosis.

Methods
Consecutive patients with carotid artery stenosis (>70% diameter stenosis on angiography) according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria and symptomatic with ipsilateral transient ischemic attack were included prospectively in this study at Huddinge University Hospital, Stockholm, Sweden, during a 9-month period.17 The institutional ethics committee approved the study, and informed consent was obtained before enrollment. Eleven patients received 40 mg/d pravastatin for an average of 3 months before CEA, whereas 13 patients did not receive any lipid-lowering therapy (control subjects). Patients in both groups received dietary counseling. Ongoing lipid-lowering medica- tion was an exclusion criterion. Fasting plasma total cholesterol, LDL, HDL, and triglyceride levels were measured at baseline and during the follow-up postoperative period with commercially avail- able assays.
TABLE 1. Baseline Characteristics of Patients in the Control and Pravastatin Groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=13)</th>
<th>Pravastatin (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61.5 (3</td>
<td>67.6 (3)</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>66</td>
<td>64</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>25</td>
<td>55</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Medication use, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>Warfarin</td>
<td>75</td>
<td>91</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>8</td>
<td>18</td>
</tr>
<tr>
<td>Diuretic agents</td>
<td>17</td>
<td>36</td>
</tr>
<tr>
<td>Oral hypoglycemic agents</td>
<td>17</td>
<td>45</td>
</tr>
<tr>
<td>Insulin</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

None of the variables listed in this table differed significantly between the 2 groups.

Tissue Preparation

After surgery, the CEA specimens were cut perpendicular to the long axis into 2 halves. The first half was snap-frozen and stored at −70°C. The second half was fixed in 4% formalin/50 mmol/L BHT/0.2% EDTA overnight at room temperature and cut into 2 halves as above, 1 of which was used for oil red O staining and the other embedded in paraffin for terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick end-labeling (TUNEL) and immunohistochemistry. Two parallel tissue sections were used from each tissue. The LDL was prepared as described by Redgrave and Carlson18 with an EDTA concentration of 10 mmol/L. EDTA was removed before oxidation by filtration on an Econo-Pac 10 DG gel column (BioRad). Protein content was determined according to Lowry et al.19 LDL (300 μg/mL) was oxidized by incubation in 10 μmol/L CuSO4/RPMI 1640 at 37°C for 18 hours and confirmed by agarose gel electrophoresis.

Immunohistochemistry

Mouse monoclonal antibodies were used for immunostaining of macrophages (CD68 KP-1, DAKO, 1:200), smooth muscle cells (SMCs; HHF-35, DAKO, 1:800), oxidized LDL (NA 59, a gift from Joseph Witzum, MD, University of California San Diego, 1:600), nuclear factor-κB (NF-κB; p65 subunit, Boehringer Mannheim, 1:200), and sheep anti–human apolipoprotein B (apoB, Boehringer Mannheim, 1:6000). Rabbit polyclonal antibody (CD3, DAKO, 1:200) was applied for detection of activated T cells. Matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinases (TIMP) immunoreactivity were determined by use of specific antibodies (Biotrak Inc). Immunostaining for adhesion molecules was performed with recombinant human vascular cell adhesion molecule-1 (rhVCAM-1) and goat anti–human intercellular cell adhesion molecule-1 (ICAM-1; R&D Systems, 1:200). For CD 3 and CD 68 antibody staining, enzymatic digestion was performed by pretreatment of sections with pronase for 5 minutes. Before immunostaining for NF-κB, sections were treated in a microwave oven in 0.1 mol/L citrate buffer, pH 6.0. Endogenous peroxidase was blocked by incubation with 0.3% H2O2 in methanol for 30 minutes. Slides were incubated with normal horse serum or 5% BSA for 30 minutes and then primary antibody for 1 hour at room temperature at the concentrations mentioned above. The apoB immunostaining was performed overnight at +4°C. Control slides were incubated with a mouse monoclonal IgG2b (Immunotech SA, 1:50) or PBS solution. The sections were incubated with the complementary secondary antibody for 30 minutes and then with avidin-biotin for 30 minutes. Sections were exposed to DAB for 3 to 5 minutes and counterstained with hematoxylin. Double immunostaining for macrophages and SMCs was used to identify the cellular source of MMP and TIMP.

Specificity of the NA 59 antibody immunostaining was confirmed by elimination of staining with preincubation of antibody with 100 μg/mL of oxidized LDL for 2 hours at 37°C before application to the tissue. The LDL was prepared as described by Redgrave and Carlson18 with an EDTA concentration of 10 mmol/L. EDTA was removed before oxidation by filtration on an Econo-Pac 10 DG gel column (BioRad). Protein content was determined according to Lowry et al.19 LDL (300 μg/mL) was oxidized by incubation in 10 μmol/L CuSO4/RPMI 1640 at 37°C for 18 hours and confirmed by agarose gel electrophoresis.

Oil Red O Staining for Lipid Content

Two parallel carotid sections from each CEA specimen were incubated in 60% isopropanol for 2 minutes and then in oil red O solution for 20 minutes and rinsed in H2O. One of the sections was counterstained with hematoxylin.

Sirius Red Staining for Collagen Content

Sirius red polarization microscopy was used to detect interstitial collagen. Collagen types I and III are identified by birefringence under polarized light illumination. Carotid plaque sections were rinsed with distilled water and incubated with 0.1% Sirius red in

TABLE 2. Lipoprotein Levels of Patients in the Control and the Pravastatin Groups at Baseline and During Follow-Up

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Chol Baseline</th>
<th>LDL Baseline</th>
<th>HDL Baseline</th>
<th>TG Baseline</th>
<th>Chol Follow-Up</th>
<th>LDL Follow-Up</th>
<th>HDL Follow-Up</th>
<th>TG Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=13)</td>
<td>6.95 ± 1.19</td>
<td>4.58 ± 1.13</td>
<td>1.39 ± 0.31</td>
<td>2.23 ± 0.87</td>
<td>6.21 ± 1.10</td>
<td>4.05 ± 1.31</td>
<td>1.36 ± 0.24</td>
<td>2.45 ± 1.57</td>
</tr>
<tr>
<td>Pravastatin (n=11)</td>
<td>6.90 ± 1.43</td>
<td>4.92 ± 1.41</td>
<td>1.23 ± 0.31</td>
<td>2.44 ± 1.67</td>
<td>5.14 ± 1.31</td>
<td>3.12 ± 1.07</td>
<td>1.19 ± 0.32</td>
<td>2.02 ± 1.34</td>
</tr>
<tr>
<td>P (control vs pravastatin)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
<td>NS*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Control group P (baseline vs follow-up)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.006</td>
<td>0.003</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Pravastatin group P (baseline vs follow-up)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.006</td>
<td>0.003</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Chol indicates cholesterol; TG, triglycerides. Values are presented as mmol/L and mg/dL in parentheses; mean ± SD.

*P=0.06.
saturated picric acid for 90 minutes. Sections were rinsed 2 times with 0.01N HCl for 1 minute and then immersed in distilled water. After dehydration with 70% ethanol for 30 seconds, the sections were observed under polarized light after coverslipping. The sections were photographed with identical exposure settings for all sections.

**Image Analysis of Immunostaining and Oil Red O Stains**

All parameters of atherosclerotic lesions were assessed by an observer blinded to treatment assignment using microimage analysis software or Image-Pro Plus software (Media Cybernetics), a BX 60 Olympus or Nikon E600 microscope, and a Spot II or Sony digital camera. Exposure times for each section were kept constant. Color segmentation was used to separate staining area from background on the basis of the color characteristics within the area of interest. For oil red O, Sirius red, apoB, NA 59, MMPs, and TIMP, the percentage of positively stained area as a function of the total plaque area was determined by computer-assisted morphometry of the plaques, and for cells expressing CD 3, CD 68, HHF-35, VCAM-1, ICAM-1, and NF-κB immunoreactivity, the percentages of positive-stained cells were measured.

**Statistical Analysis**

Data are presented as mean±SD. Unpaired t test was used to compare the 2 groups, and changes in lipid values in each group from baseline to follow-up were tested with a paired t test. A value of P<0.05 was considered statistically significant.

**Results**

Clinical characteristics and baseline lipoprotein levels were comparable in the 2 groups (Tables 1 and 2). In control subjects, lipoprotein levels did not change significantly during follow-up, whereas total cholesterol decreased by 27% and LDL cholesterol decreased by 34% in the pravastatin group (Table 2).

Carotid plaques of both groups had the morphology of advanced lesions, with a lipid-rich acellular core and sites of rupture, superimposed thrombosis, and intraplaque hemorrhage.

**Lipid Content, Oxidized LDL, and ApoB-100 Immunoreactivity**

The lipid content by oil red O and the oxidized LDL content were significantly lower in the plaques of the pravastatin-treated group than in control subjects. ApoB-100 content, however, was comparable between the groups (Figure 1, Table 3).

**Cellular Composition, Adhesion Molecules, NF-κB, and Cell Death**

Plaques from the pravastatin-treated group had reduced immunoreactivity for macrophages (CD 68) and T cells (CD 3). Reduced ICAM-1 and VCAM-1 in the pravastatin group was

<table>
<thead>
<tr>
<th>TABLE 3. Lipid Content in Carotid Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Control (n=13)</td>
</tr>
<tr>
<td>Pravastatin (n=11)</td>
</tr>
</tbody>
</table>

NA59 is oxidized LDL. Values represent percentage positive-stained lesional area; data are mean±SD.
not statistically different between the groups. The fraction of cells with positive immunoreactivity for SMC α-actin was slightly higher in the pravastatin group than in control subjects. NF-κB immunoreactivity was similar between the 2 groups (Figure 2, Tables 4 and 5).

The TUNEL assay identifies cells with DNA fragmentation. Compared with the control subjects, the fraction of TUNEL-positive cells was reduced by ≈50% in the pravastatin group. Most of the apoptotic cells were identified as SMCs in serial sections.

**MMP, TIMP, and Collagen Content**

Plaques from the pravastatin group had less MMP-2 and increased TIMP-1 immunoreactivity (Figures 3 through 5 and Table 6). The majority of the cells expressing MMP-2 were macrophages with weak expression by SMCs, as identified by morphological features, colocalization in contiguous sections (Figure 4A), and double immunostaining (Figure 4C). Macrophages but not α-actin–reactive SMCs were shown to express TIMP-1 on contiguous sections (Figure 4B) and on double immunostaining. TIMP-1 immunoreactivity, however, exceeded macrophage immunoreactivity in the pravastatin-treated group, suggesting that non–α-actin–reactive SMCs may have been an additional source of increased TIMP-1 immunoreactivity in the pravastatin group. There was no significant difference in MMP-1, MMP-9, and TIMP-2 immunoreactivity between the 2 groups. Collagen content was significantly higher in the pravastatin-treated group than in control subjects (Figure 5).

**Discussion**

This study shows that pravastatin treatment for 3 months is associated with reduced lipid content, oxidized LDL immunoreactivity, cell death, and inflammatory cell and MMP-2 immunoreactivity, along with an increase in TIMP-1 immunoreactivity and interstitial collagen content in human carotid plaques. We did not detect a significant change in MMP-1, MMP-9, and TIMP-2 expression, for reasons unclear to us, although more prolonged therapy may be necessary for these changes to occur, as suggested by experimental data. Despite the lack of a significant decrease in MMP-1 and MMP-9 immunoreactivity, increased collagen accumulation may nevertheless have resulted from an increase in TIMP-1, a potent inhibitor of both MMP-1 and MMP-9 activity, and reduced MMP-2 levels. Selective overexpression of MMP-2, capable of degrading basement membrane type IV collagen, has been shown in the proteoglycan-rich matrix underlying plaque erosion, where it has been implicated in loss of endothelial anchorage to the underlying matrix. Thus, a reduction in MMP-2 may also contribute to plaque stability by reducing plaque erosion, which has been shown to be a substrate for coronary thrombosis in ≥50% fatal events. Reduction in MMP expression observed in this study may have resulted from a decrease in the number of macrophages, as noted in this study, and/or reduced gene transcription/activation of MMPs. Pravastatin treatment resulted in substantially lower oxidized LDL immunoreactivity, which may have resulted from either reduced vessel wall lipid retention or direct antioxidant effects of pravastatin. A reduced lipid content of plaques, along with a reduction in oxidized LDL immunoreactivity, also suggests that decreased lipid accumulation and reduced oxidative stress may have contributed to a reduction in inflammation and TIMP-1 increase and MMP-2 reduction in the vessel wall, because LDL oxidation is known to activate the inflammatory cascade and regulate TIMP-1 and MMP expression. The present findings indicate that pravastatin may affect plaque lipid metabolism through inhibition of oxidative modification of LDL in the vessel wall and favor removal by normal pathways. The observation that pravastatin-treated lesions contained equal amounts of apoB-100 despite decreased lipid content may thus be due to decreased oxidation-dependent fragmentation of apoB-100.

Clinical trials have shown that lipid-lowering therapy results in substantially greater reduction in cardiovascular events than would be expected from modest angiographic changes. The protective effect is also evident long before effects on lesion progression are detectable. These observations suggest the possibility that the beneficial effects of lipid lowering may result from mechanisms other than reduction of plaque size or stenosis severity. Because plaque disruption leading to thrombosis is a major cause of acute cardiovascular events, a reduced frequency of plaque disruption through increased stability represents one such possible mechanism. The vulnerability of plaques to disruption appears to be related to plaque composition as well.

**TABLE 4. T-Cell, Macrophage, SMC, and TUNEL Positivity in Carotid Lesions**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>CD3</th>
<th>P</th>
<th>CD68</th>
<th>P</th>
<th>HHF35</th>
<th>P</th>
<th>TUNEL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=13)</td>
<td>24.3±13.4</td>
<td>0.25</td>
<td>25.3±12.5</td>
<td>0.05</td>
<td>14.3±12.4</td>
<td>&lt;0.05</td>
<td>32.0±12.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pravastatin (n=11)</td>
<td>11.2±9.3</td>
<td>&lt;0.05</td>
<td>15.0±10.2</td>
<td>&lt;0.05</td>
<td>16.9±13.8</td>
<td>NS</td>
<td>17.7±7.8</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

CD3 is T cells; CD68, macrophages; HHF35, SMCs; and TUNEL, apoptotic cells. Values represent percentage positive cells; data are mean±SD.

**TABLE 5. Adhesion Molecules and Transcription Factor NF-κB in Carotid Lesions**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>VCAM-1</th>
<th>P</th>
<th>ICAM-1</th>
<th>P</th>
<th>NF-κB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=13)</td>
<td>9.4±5.5</td>
<td>0.25</td>
<td>10.2±5.9</td>
<td>0.05</td>
<td>14.1±7.9</td>
<td>NS</td>
</tr>
<tr>
<td>Pravastatin (n=11)</td>
<td>8.0±2.8</td>
<td>NS</td>
<td>8.2±3.6</td>
<td>NS</td>
<td>11.1±4.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values represent percentage positive cells; data are mean±SD.
as hemodynamic factors. Although advanced atherosclerotic lesions are heterogeneous in nature, the majority are characterized by a large lipid-rich core and a thin fibrous cap with increased numbers of inflammatory cells in the shoulder region of the plaque.27 Several studies suggest that lipoproteins may contribute to the development of atherosclerosis by initiating and sustaining the inflammatory response in the vessel wall.24 Increased inflammation is also a feature of disrupted plaques. There is a strong positive correlation between concentration of macrophages localized at sites of intimal rupture and acute coronary syndromes.27–29 The secretion of matrix-degrading enzymes, ie, MMPs, largely by macrophages, may cause disruption of the plaque structure by depleting the fibrous cap of connective tissue matrix.30–32 Recent reports indicate that elevated levels of C-reactive protein (CRP), an acute-phase protein widely used as a marker of inflammation, is predictive of the risk of first myocardial infarction.33 The severity of the superficial inflammation seen in atherosclerotic lesions has been implicated as a statistically significant correlate of plaque rupture.29,34

It remains to be determined whether the decrease in inflammatory activity observed in plaques from the pravastatin group is due to a decreased presence of oxidized lipids. Recent data from subgroup analysis of the CARE study suggest that the beneficial clinical effects of pravastatin treatment in post–myocardial infarction patients with increased markers of inflammation may result in part from anti-inflammatory as well as lipid-lowering properties.35,36 These observations suggest that pravastatin may have anti-inflammatory effects that are independent of lipid-lowering effects.15,35–37

The vulnerable lesion characteristically has a thin fibrous cap with few SMCs, many of which show signs of death by apoptosis or oncosis (necrosis), which can be identified by TUNEL staining.38,39 The lack of functional SMCs could lead to decreased collagen content, thereby reducing fibrous cap tensile strength.39 We found reduced cell death (of mostly SMCs) in carotid plaques of pravastatin-treated patients, which may either have resulted from a direct effect of the drug or have been an indirect consequence of reduced LDL oxidation in the plaque.40 Because a change in phenotype may change α-actin expression by SMCs, α-actin staining may not have identified all SMCs, thereby accounting for the lack of significant difference in SMC numbers between the 2 groups. Further research is needed, however, to confirm this observation.

Conclusions

This study demonstrates that pravastatin-induced lipid-lowering therapy is associated with changes in human carotid plaque composition that favor lesion stability, providing the first strong evidence in support of plaque-stabilizing effects of statins in humans.

Potential Limitations of the Study

The results of this study must be considered with the following caveats: this was a nonrandomized study, and hidden biases could explain the results. This is unlikely, however, because patients were consecutive and well matched for various demographic features. Although differences in composition were clearly demonstrated, the possibility of sampling error should also be considered. We did not measure the true mechanical properties of the plaques to ascertain whether pravastatin-treated plaques were indeed more resistant to rupture, although one could infer that from an increase in collagen content and a reduction in lipid content and inflammation, all of which appear to relate to the in vitro tensile strength of plaques.41–43

Although favorable changes in lesion composition were observed within 3 months of pravastatin treatment, clinical trials suggest that event reduction takes 6 to 12 months after initiation of statins.1–5 This seeming discrepancy may result from the fact that a more complete lipid and inflammatory cell depletion is necessary to completely stabilize plaques.

### TABLE 6. MMP, TIMP, and Collagen Content

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Collagen</th>
<th>P</th>
<th>MMP-1</th>
<th>P</th>
<th>MMP-2</th>
<th>P</th>
<th>MMP-9</th>
<th>P</th>
<th>TIMP-1</th>
<th>P</th>
<th>TIMP-2</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=13)</td>
<td>7.5±3.5</td>
<td></td>
<td>10.4±4.7</td>
<td>8.4±5.3</td>
<td>3.7±3.7</td>
<td>3.1±3.9</td>
<td>1.2±0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pravastatin (n=11)</td>
<td>12.4±3.1</td>
<td>0.003</td>
<td>8.2±5.0</td>
<td>NS</td>
<td>3.6±3.9</td>
<td>0.03</td>
<td>3.7±2.8</td>
<td>NS</td>
<td>9±6.2</td>
<td>0.02</td>
<td>1.9±2.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as percentage of plaque area, mean±SD.
Figure 4. Contiguous sections show that MMP-2 colocalizes with macrophage immunoreactivity (A, a and b) and weakly with SMC immunoreactivity (A, c and d), whereas TIMP-1, while colocalizing with macrophage immunoreactivity, also appears to exceed area of macrophage immunoreactivity but not to colocalize with SMC (α-actin) immunoreactivity (B, a through d). These findings are further confirmed by double immunostaining for macrophages (red) and MMP-2 (brown) in C, a; macrophages (red) and TIMP-1 (brown) in C, b; SMCs (red) and MMP-2 (brown) in C, c; and SMCs (red) and TIMP-1 (brown) in C, d (see text for details).
and such effects require more prolonged therapy. Nevertheless, our study shows that plaque composition is changing in the appropriate direction, and the findings do not conflict with the results of clinical trials.

Acknowledgments
This study was supported by grants from the Swedish Medical Research Council, the Swedish Heart and Lung Foundation, Lars Hjertas Minne’s Foundation, and Bristol-Myers Squibb. The support of the Dora Herbert Family and Spaulding Family (to Dr Shah) is gratefully acknowledged. The authors express their sincere gratitude to Dr Björn Wiklund and Dr Rolf Pieper from the Department of Vascular Surgery, Huddinge University Hospital, for providing the carotid specimens.

References


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_Circulation_. 2001;103:926-933
doi: 10.1161/01.CIR.103.7.926

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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