Intensive Treatment With Atorvastatin Reduces Inflammation in Mononuclear Cells and Human Atherosclerotic Lesions in One Month

Jose Luis Martín-Ventura, PhD; Luis Miguel Blanco-Colio, PhD; Almudena Gómez-Hernández, PhD; Begoña Muñoz-García, BsC; Melina Vega, MD; Javier Serrano, MD; Luis Ortega, MD; Gonzalo Hernández, MD; José Tuñón, MD; Jesús Egido, MD

Background and Purpose—To investigate the effect of short-term high-dose atorvastatin on blood and plaque inflammation in patients with carotid stenosis.

Methods—Twenty patients undergoing carotid endarterectomy without previous statin treatment were randomized to receive either atorvastatin 80 mg/d (n=11) or no statins (n=9) for 1 month. We studied inflammatory mediators in plasma (enzyme-linked immunosorbent assay), peripheral blood mononuclear cells (PBMCs; quantitative RT-PCR and EMSA) and plaques (immunohistochemistry and Southwestern histochemistry).

Results—Atorvastatin significantly decreased total and low-density lipoprotein cholesterol and prostaglandin E2 plasma levels. PBMCs from treated patients showed impaired NF-κB activation and MCP-1 and COX-2 mRNA expression. Carotid atherosclerotic plaques demonstrated a significant reduction in macrophage infiltration, activated NF-κB, and COX-2 and MCP-1 expression.

Conclusions—Intensive treatment with atorvastatin decreases inflammatory activity of PBMCs and carotid atherosclerotic plaques in 1 month. These data strongly suggest that the antiinflammatory effect of high doses of statins in humans can be seen very early. (Stroke. 2005;36:1796-1800.)

Key Words: atherosclerosis ■ blood ■ carotid arteries ■ inflammation

Atherosclerosis results from passive lipid deposition in the vascular wall, inducing an active inflammatory process. Nuclear factor-κB (NF-κB) is activated in the vulnerable region of human atherosclerotic plaques and in peripheral blood mononuclear cells (PBMCs) of patients with carotid atherosclerosis.1 This transcription factor regulates the expression of monocyte chemoattractant protein-1 (MCP-1) and cyclooxygenase-2 (COX-2), an inducible enzyme that enhances the production of the chemoattractant prostaglandin E2 (PGE2).

Clinical trials with statins demonstrated a marked reduction in cardiovascular mortality, probably in part, by modulating the inflammatory component of plaques.2 Intensive statin treatment reduces endothelial dysfunction and thrombotic events in short periods of treatment.3,4 We analyzed whether treatment with high doses of atorvastatin decrease inflammation in PBMCs and carotid atherosclerotic plaques in 1 month in patients with carotid stenosis ≥70% without previous statin treatment.

Methods

Patients with inflammatory or neoplastic disease, major surgery, or myocardial infarction in the previous 6 months, or those receiving antiinflammatory drugs (except acetylsalicylic acid up to 325 mg/d) or statins in the previous year were excluded. Informed consent was obtained, blood was drawn, and patients were allocated by using a random number table to receive either atorvastatin 80 mg/d (n=11) or no statin (n=9) until scheduled carotid endarterectomy was performed. At surgery, blood samples and atherosclerotic plaques were collected. The study was approved by the local ethical committee.

Blood Studies

Total cholesterol, low-density lipoproteins, triglycerides, and high-density lipoproteins were measured by enzymatic assays (Sigma), and MCP-1 and PGE2 plasma levels were measured by enzyme-linked immunosorbent assay (R&D Systems).

PBMCs were obtained from blood drawn at randomization and at the time of surgery.1 Protein extracts were prepared for electrophoretic mobility shift assay.1 Quantification was performed by densitometric analysis (ImageQuant program), yielding results in arbitrary units.
Total RNA was extracted, quantitative reverse-transcription polymerase chain reaction was performed, and target genes were corrected for GADPH.

**Plaque Studies**

Specimens were collected and processed using monoclonal anti-human macrophages (HAM-56; DAKO), COX-2 (Cayman), and MCP-1 (Abcam) antibodies. Negative controls using the corresponding IgG were included to check for nonspecific staining. In situ NF-κB activity was detected by Southwestern histochemistry. Competition assays with 200-fold excess of unlabeled probe were used as negative controls.

Morphometry was performed with the Olympus semiautomatic image analysis system. Results are expressed as percentage of positive staining/mm² (immunohistochemistry) and nuclei staining positive/mm² (Southwestern).

**Statistical Analysis**

Statistical analysis was performed with GraphPAD InStat (Graph-PAD Software). Lipid levels and enzyme-linked immunosorbent assay data were presented as median (interquartile range) and analyzed by the Kruskal–Wallis test. Changes in lipids and enzyme-linked immunosorbent assay levels in every treatment group were assessed by a covariance analysis using the basal levels as the covariate. EMSA, quantitative polymerase chain reaction, and Southwestern and immunohistochemistry data at the end of the study were presented as mean ± 95% confidence interval and analyzed by Mann–Whitney test. Categorical variables were assessed by Fisher’s exact test. Differences were considered significant at 2-tailed P<0.05.

**Results**

Clinical data were well-balanced between both groups. Atorvastatin reduced total and low-density lipoprotein cholesterol and PGE₂ plasma levels, whereas no significant changes were observed in the nontreated group (Table).

In PBMCs, atorvastatin lowered NF-κB activation (1.3 [0.7 to 1.9] versus 2.8 [1.5 to 3.9]; P<0.05) and MCP-1 and COX-2 mRNA expression (0.5 [0.1 to 0.8] versus 3.7 [0.7 to 6.8] and 1.7 [0.5 to 2.9] versus 3.3 [1.2 to 5.3], respectively; P<0.05 for both; Figure 1). No significant differences were noted between these parameters of both

**Characteristics of Patients**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=9)</th>
<th>ATV (n=11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>70 (63–76)</td>
<td>69 (59–77)</td>
<td>0.79</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6 (66%)</td>
<td>9 (82%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Female</td>
<td>3 (33%)</td>
<td>2 (18%)</td>
<td>0.62</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3 (33%)</td>
<td>4 (36%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (78%)</td>
<td>11 (100%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Present smoker</td>
<td>1 (11%)</td>
<td>2 (18%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>2 (22%)</td>
<td>4 (36%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Lower limb ischemic disease</td>
<td>5 (55%)</td>
<td>5 (45%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>2 (22%)</td>
<td>2 (18%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>3 (33%)</td>
<td>3 (27%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>1 (11%)</td>
<td>0 (0%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>...</td>
</tr>
<tr>
<td>Thienopyridine</td>
<td>3 (33%)</td>
<td>3 (27%)</td>
<td>1.00</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>5 (55%)</td>
<td>7 (64%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium-channel blockers</td>
<td>2 (22%)</td>
<td>3 (27%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Diuretics</td>
<td>1 (11%)</td>
<td>0 (0%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Oral hypoglycemic drugs</td>
<td>1 (11%)</td>
<td>1 (9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Insulin</td>
<td>1 (11%)</td>
<td>1 (9%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

*P<0.005 vs control (after); †P=0.016; ‡P=0.038; §P=0.010 vs ATV (before).
groups at baseline. Changes in total and differential leukocyte counts were not significantly different between groups.

Atorvastatin impaired plaque macrophage infiltration (2.5 [0.1 to 5.2] versus 9.3 [3.1 to 15.5]%; \(P < 0.05\)), NF-kB activation (5706 [4865–6538] versus 8063 [6219–9923] positive nuclei/mm\(^2\); \(P < 0.05\)), and MCP-1 and COX-2 expression (11 [9–14] versus 24 [14–33]% and 16 [11–20] versus 34 [24–43]%, respectively; \(P < 0.05\) for both) (Figure 2).

**Discussion**

We show that atorvastatin 80 mg/d diminishes lipid levels and blood inflammatory mediators (PGE\(_2\) plasma levels, NF-\(\kappa\)B activity, and MCP-1 and COX-2 expression in PBMCs) in patients with carotid atherosclerosis within 1 month.

Atorvastatin 80 mg/d decreased macrophage infiltration in plaques as shown after 3 to 4 months of moderate statin treatment.\(^5\)\(^\sim\)\(^6\) Moreover, NF-\(\kappa\)B activation, MCP-1, and COX-2 expression were also reduced. This may have been responsible for the reduction in macrophage infiltration, as shown in animal models.\(^7\) Of interest, 40 mg/d pravastatin for 3 months did not affect NF-\(\kappa\)B activation in carotid atherosclerotic plaques.\(^5\) This suggests that intensive statin treatment has a faster antiinflammatory effect than moderate doses, in accordance with the decrease in atherosclerosis progression observed with high-dose atorvastatin in a recent clinical trial.\(^8\) Nevertheless, our data must be interpreted cautiously, given the small sample size and that it was not a placebo-controlled study.

In conclusion, our findings suggest that intensive treatment with atorvastatin decreases the inflammatory activity of human PBMCs and carotid plaques in 1 month.
Acknowledgments
This work was supported by grants from cardiovascular network (03/01), Fundación Española del Corazón, SAF 2004/0619, CAM (GR/SAL/0411/2004), European network (QLG1-CT-2003-01215), and Pfizer-Spain (Beca Pfizer SEA 2001).

References


Intensive Treatment With Atorvastatin Reduces Inflammation in Mononuclear Cells and Human Atherosclerotic Lesions in One Month

Jose Luis Martín-Ventura, Luis Miguel Blanco-Colio, Almudena Gómez-Hernández, Begoña Muñoz-García, Melina Vega, Javier Serrano, Luis Ortega, Gonzalo Hernández, José Tuñón and Jesús Egido

Stroke. 2005;36:1796-1800; originally published online July 14, 2005; doi: 10.1161/01.STR.0000174289.34110.b0

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/36/8/1796

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org/subscriptions/