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EXPERIMENTAL STUDIES

HMG-CoA Reductase Inhibition by Atorvastatin Reduces Neointimal Inflammation in a Rabbit Model of Atherosclerosis

CARMEN BUSTOS, PHD, MIGUEL A. HERNÁNDEZ-PRESA, PHD, MÓNICA ORTEGO, BS, JOSÉ TUÑÓN, MD, LUIS ORTEGA, MD,* FERNANDO PÉREZ, PHD,† CRISTINA DÍAZ, MD,‡ GONZALO HERNÁNDEZ, MD,‡ JESÚS EGIDO, MD

Madrid and Barcelona, Spain

Objectives. **To study the effect of the 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA)-reductase inhibitor atorvastatin on the potential mechanisms involved in the recruitment of monocytic cells into the vessel wall.**

Background. **Inhibitors of HMG-CoA-reductase reduce cardiovascular mortality though the mechanisms yet elucidated. Most ischemic events are secondary to disruption of atherosclerotic plaques highly infiltrated by macrophages.**

Methods. **Atherosclerosis was induced in the femoral arteries of rabbits by endothelial damage and atherogenic diet for 4 weeks. Then, animals were switched to standard chow and randomized to receive either no treatment or atorvastatin (5 mg/kg/d) and killed after 4 weeks.**

Results. **Atorvastatin induced a significant reduction in serum lipids and in lesion size. Arterial macrophage infiltration was abolished by the treatment, and monocyte chemoattractant protein-1 (MCP-1) was significantly diminished in the neointima and in the media. Nuclear factor kappa-B (NF-κB) was activated in the 60% of the lesions, both in macrophages and vascular smooth muscle cells (VSMC), of the untreated group while only in 30% of the atorvastatin group. NF-κB activity was also lower in the uninjured aorta and liver of treated compared with untreated rabbits. In cultured VSMC, MCP-1 expression and NF-κB activity induced by tumor necrosis factor alpha were downregulated by atorvastatin.**

Conclusions. **In a rabbit atherosclerosis model, atorvastatin diminishes the neointimal inflammation, and this could contribute to the stabilization of the atherosclerotic plaque. This may be an additional explanation for the reduction of acute ischemic events in patients treated with statins.**

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Cholesterol-lowering drugs slow the progression of coronary atherosclerosis and diminish the incidence of ischemic events (1). Moreover, the inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA)-reductase also reduce total and coronary mortality, both in secondary and in primary prevention (2,3).

Acute ischemic events are mainly secondary to plaque disruption and thrombosis. Although it is well known that lipid-rich plaques are more prone to rupture, the mechanisms by which HMG-CoA-reductase inhibitors reduce coronary events are not completely understood. Macrophage infiltration has been related to unstable coronary syndromes, probably because these cells secrete proteolytic enzymes, which weaken the cap of the lesion (4,5). As a consequence, the study of the mechanisms leading to the accumulation of macrophages within the lesions is reaching increasing interest. Monocyte chemoattractant protein-1 (MCP-1) is the best studied attractant for monocytes, and its presence in the neointima has been demonstrated in several experimental models and in humans (6). Nuclear factor kappa-B (NF-κB) is the main nuclear factor involved in the activation of its transcription (7), and we have recently shown that this factor was activated in the damaged arteries in a model of atherosclerosis in rabbits (8).

The purpose of this study was to determine the effect of the HMG-CoA-reductase inhibitor atorvastatin on the neointimal content of macrophages, the presence of MCP-1 and the NF-κB activation in an experimental model of atherosclerosis in rabbits. In addition we explored whether atorvastatin could modulate in a direct manner these factors in cultured VSMC.

Methods

In Vivo Study. *Design.* Twenty-five New Zealand male rabbits weighing 3.5–4 kg (mean 3.4 ± 0.6 kg) were used (Fig. 1). The animals were housed in individual cages and quarantined for 7 days before use, and were treated according to the Fundación Jiménez-Díaz's policies guidelines for animal care. Atherosclerosis was induced in each femoral artery by endothelial desiccation with nitrogen, as previously described (9),

From the Fundación Jiménez Díaz, Universidad Autónoma; *Hospital Clínico and †Hospital Gómez Ulla, Universidad Complutense, Madrid; and ‡Parke Davis Spain, Barcelona, Spain. This work was supported in part by grants from CICYT (SAF97/55 and FIS98/1269), Fundación Renal Iñigo Alvarez de Toledo and Parke Davis.

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Address for correspondence: Dr. Jesús Egido, Research Laboratory, Fundación Jiménez Díaz, Avda. Reyes Católicos 2. 28040 Madrid, E-mail: evasc@uni.fjd.es.

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Abbreviations and Acronyms

AP-1	=	activated protein-1
G3PDH	=	glyceraldehyde 3-phosphate dehydrogenase
HMG-CoA	=	3-hydroxy-3-methyl-glutaryl-coenzyme A
MCP-1	=	monocyte chemoattractant protein-1
NF-κB	=	Nuclear factor kappa-B
TNFα	=	tumor necrosis factor alpha
VSMC	=	vascular smooth muscle cells

followed by 4 weeks of atherogenic diet (0.5% cholesterol and 14% coconut oil diet) (Mucedola, Italy). After that, a control angiography was performed to discard those animals with a complete occlusion of the artery, and they were switched to standard chow and randomized to receive 5 mg/kg/d of atorvastatin (Parke-Davis, Ann Arbor, MI) (n = 7) or no treatment (n = 9). The dose of atorvastatin was chosen on the basis of previous experiments designed to test the effect of the drug (1, 2, 5 and 10 mg/kg/day) on chow consumption. The control of the drug intake was done daily and the dietary regime consisted of feeding 50 g of standard chow the first week, 100 g the second week and 150 g the last 2 weeks. After 4 weeks all animals were killed. In Figure 1, a scheme of the experimental protocol is shown. Five control animals fed standard chow and with no experimental intervention were also studied.

Angiography. Animals were anesthetized and given antibiotics. After the medial laparotomy, the abdominal aorta was reached and exposed. A ligature was placed to control the bleeding, and then it was cannulated and nitroglycerin was infused to avoid spasm. After 1 min, and under microscope, 2 mL of contrast (Sodium Ioxaglato, Hexabris 320; ROVI S.A., Madrid, Spain) was infused. Hemostasis was achieved by local pressure and the wound was closed. Four animals with an occluded artery were excluded from the study.

Sample collection. Animals were anesthetized and both femoral arteries and the aorta were exposed. One of the femoral arteries and a piece of the aorta were removed, the adventitial layer was carefully peeled off and immediately snap-frozen in liquid nitrogen. The animals were killed with an overdose of pentobarbital (Abbot, Madrid, Spain) and a liver sample was obtained and frozen. The other femoral artery was cannulated, fixed in situ with 4% buffered formaldehyde at 100 mm Hg pressure, removed and embedded in paraffin. Plasma samples were collected 24 h postmeal at the beginning of the study, at the moment of randomization and at death. Plasma cholesterol, LDL and HDL cholesterol and triglycerides were measured by enzymatic techniques (bioMérieux, Lyon, France).

Morphometric analysis. The morphometric analysis was performed on orcein-stained arterial sections with the NewSketch 1212 graphic tablet (Genius, Ontario, Canada) linked to a microcomputer using the autoCAD 10.0 software. Sections with the maximal lesion were chosen for quantification.

Immunohistochemistry. Paraffin-embedded arteries were cross-sectioned into 4-μm-thick pieces at 5-mm intervals from the proximal to the distal end, dewaxed and rehydrated. Macrophages and MCP-1 were identified according to previously described techniques (8).

For quantification, the sections with the maximal lesion in each animal were chosen. Computer-assisted morphometric analysis was performed with the Cue-2 semiautomatic image analysis system (Olympus, Hamburg, Germany). The arterial cross-sections stained with the antibodies were digitized via an Olympus microscope (BH-2) connected to a CCD video camera. The labeled areas in the intima and the media were delimited, and after image enhancement and segmentation (transformation to a binary image) a grey value ranging from 0 to 255 was assigned to each pixel and automatic analysis was performed. Results were expressed as immunostained area.

Southwestern histochemistry. This method was developed to detect the distribution and DNA-binding activity of NF-κB in situ using a digoxigenin-labeled double-stranded DNA probe with a specific consensus sequence that binds to NF-κB, according to a previously described technique with modifications (10). The synthetic sense (5′-AGTTGAGGGGACTTTCCCAGGC-3′) and antisense (5′-GCCTGGGAAAGTCCCCTCAACT-3′) probes (Genosys Biotechnology, London, UK) were annealed by heating at 80°C for 2 min. The probes were labeled with digoxigenin (DIG oligonucleotide 3′-end labeling; Boehringer Mannheim, Mannheim, Germany). Arterial sections were dewaxed, rehydrated, incubated

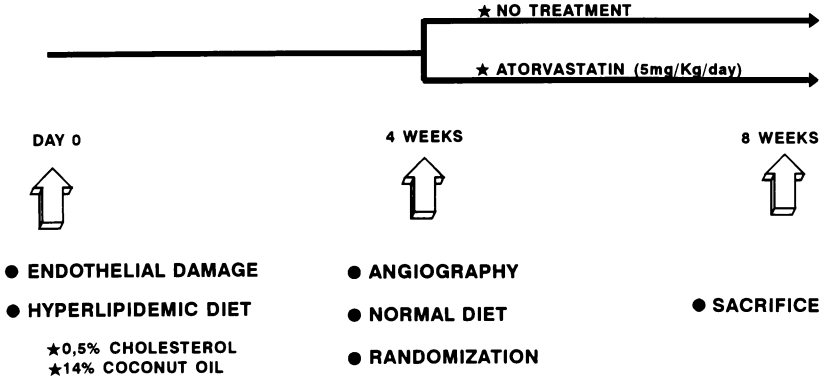


Figure 1. Scheme of the experimental protocol.

with 5 mmol/L levamisole for 30 min and fixed with 0.2% paraformaldehyde. They were then digested with 0.5% pepsin in 1N HCl for 30 min, washed with HEPES and incubated with 0.1 mg/mL DNAse I for 30 min and washed again with HEPES buffer. The DNA-binding reaction was performed by incubation with 100 pmol/mL of the labeled DNA probe overnight at 37°C, and then the preparations were washed with HEPES buffer, washing buffer (0.3% Tween 20 in: 0.1 mol/L maleic acid, 0.15 mol/L NaCl, pH 7.5) and blocking solution (0.1 × SSC, 0.1% SDS diluted 1:10 in washing buffer) for 1 h. Preparations were incubated with an anti–digoxigenin antibody conjugated with alkaline phosphatase (Boehringer Mannheim), and color reaction was developed. Sections were mounted with glycerol. Preparations without probe were used as a negative control, and to test the specificity of the technique, a mutant NF-κB probe was used (5′-AGTTGAGGCTCCTTT-CCCAGGC-3′). For the simultaneous detection of NF-κB and macrophages or VSMC, before mounting the sections, they were washed with phosphate-buffered solution (PBS) and immunohistochemistry was done according to the above-described technique for macrophages. The detection of VSMC was done with an anti-alpha-actin (HHF35; ENZO Diagnostic, New York, New York) in the same conditions as for macrophages.

Electrophoretic mobility shift assays. Tissue and nuclear protein extraction was done with modifications as previously described (8). Protein concentration was quantified by the BCA method (Pierce, Rockford, IL). Gel shift assays were performed with a commercial kit according to the instructions of the manufacturer (Promega, Madison, WI). NF-κB (5′-AGTTGAGGGGACTTTCCCAGGC-3′) and AP-1 (5′-CGCTTGATGAGTCAGCCGGAA-3′) consensus oligonucleotides were [^{32}P] end-labeled by incubation for 10 min at 37°C with 10 U T4 polynucleotide kinase (Promega) and 10 μCi [γ-^{32}P]ATP (3000 Ci/mmol) (Amersham, Buckinghamshire, United Kingdom).

In Vitro Studies. *Cell culture.* Rat thoracic aortic VSMC were isolated and cultured as previously described (8) and characterized by positive alpha-actin staining. VSMC were growth-arrested by incubation in 0.5% fetal calf serum (FCS) medium for 48 h, and then incubated with 100 U/mL TNFα (Immugenex). Atorvastatin (10^{-7} mol/L) (Sodium salt) was added to the culture medium 1 h before the stimulation.

RNA extraction and Northern blot analysis. RNA was obtained by the acid guanidine-thiocyanate-phenol-chloroform method (11) and quantified by absorbance at 260 nm in duplicate. Northern blot analysis was performed as described elsewhere (8). The cDNA probes for human MCP-1 and G3PDH, used as internal control, were obtained from the American Type Culture Collection, Bethesda, Maryland. Films were scanned using the Image Quant densitometer (Molecular Dynamics, Sunnyvale, California).

Statistical Analysis. Results are expressed as mean ± SD. Lipid values and morphometric analysis were compared by means of the Mann-Whitney U test. The macrophage infiltration was expressed as percentage and compared by the Fisher exact test. The areas immunostained positive for MCP-1 in the intima and the media of the vessel wall in treated and nontreated animales were analyzed by a Kruskal-Wallis test followed by Dunn's multiple comparison test. Significance was established with GraphPAD InStat (GraphPAD Software) and differences were considered significant when $p < 0.05$ (two-tailed).

Results

Weight and plasma lipids evolution. The weight of the animals was controlled weekly to adjust the dose of drug. Atorvastatin treatment did not modify weight evolution (no treatment: 3.21 ± 0.75 kg vs. atorvastatin: 3.37 ± 0.72 kg). After 4 weeks on the atherogenic diet, total plasma cholesterol and LDL cholesterol raised about 20-fold over the initial values (from 54 ± 43 to 1000 ± 419 and from 42 ± 63 to 907 ± 445 mg/dL, respectively; $p < 0.004$), the triglycerides around twofold (from 63 ± 18 to 133 ± 78 mg/dL; $p < 0.004$) while the HDL cholesterol was not modified (from 25 ± 16 to 27 ± 12). When compared with the untreated animals at death, the atorvastatin group showed significantly lower levels of plasma cholesterol (249 ± 322 vs. 595 ± 404 mg/dL; $p < 0.04$), LDL (48 ± 27 vs. 79 ± 29 mg/dL; $p < 0.02$) and triglycerides (51 ± 33 vs. 96 ± 28 mg/dL; $p < 0.02$). HDL cholesterol was not modified with the treatment (25 ± 7 vs. 25 ± 9).

Analysis of the vascular lesions. The lesions were evaluated on histological preparations stained with orcein (Fig 2, top). The mean lesion size in each group was calculated from those preparations showing the maximal lesion in each animal. Maximal stenosis of the femoral arteries of the atorvastatin group was significantly lower than that of the untreated group, estimated both as intima/media ratio (0.129 ± 0.038 vs. 0.412 ± 0.322; $p = 0.001$) or as a percentage of the luminal area occupied by the lesion (10.06 ± 7.03% vs. 21.24 ± 13.54%; $p = 0.038$) (Fig. 2, bottom).

Evaluation of the presence of macrophages in the lesions. Macrophages were present in the neointima of the arteries of five out of the nine animals with no treatment (56%) and in none (0%) of the seven animals treated with atorvastatin ($p = 0.034$). The mean neointimal area occupied by macrophages was 1470 ± 2750 μm^2 in the untreated group. Macrophages were also present in the media of three out of the nine untreated animals (33%) but in none of those treated with atorvastatin (Fig. 3).

Evaluation of MCP-1 protein expression in the lesions. MCP-1 was evaluated by immunohistochemistry (Fig. 4, top). All animals in the untreated group showed staining for MCP-1 (100%) versus five out of seven in the atorvastatin group (71%). The mean area stained for MCP-1 was significantly lower in the atorvastatin compared with the untreated group, both in the neointima (3881 ± 3898 vs. 41,657 ± 43,141 μm^2; $p < 0.01$) and in the media (1027 ± 902 vs. 16,584 ± 15,401 μm^2, $p < 0.05$) (Fig. 4, bottom). No control animal showed any staining.

Macrophages and MCP-1 were colocalized in the neointima, although MCP-1 seems to be present also in the medial

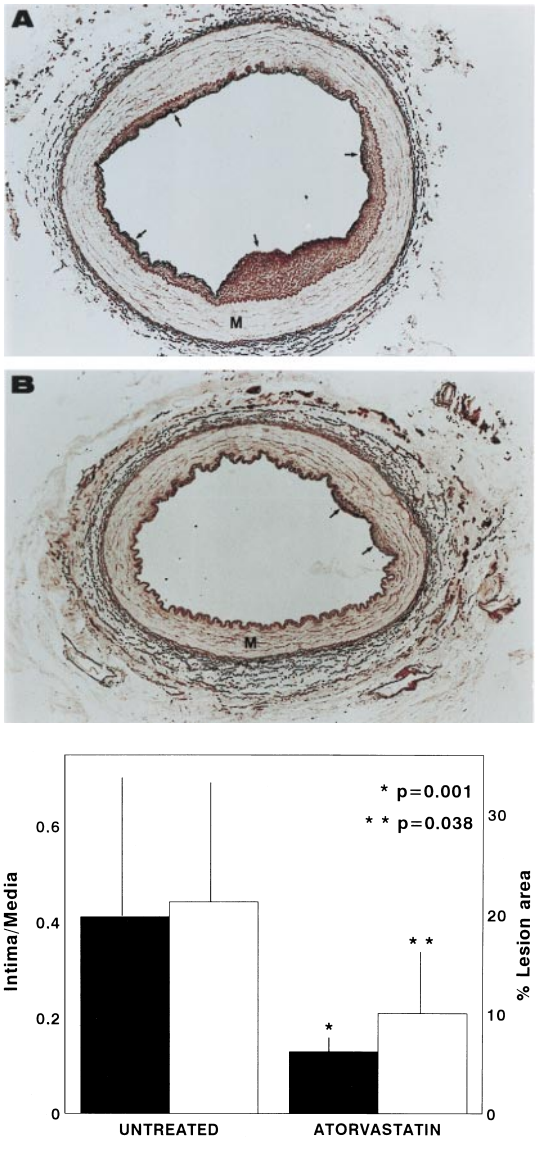


Figure 2. Analysis of the vascular lesions. **Top,** Photomicrographs of representative orcein-stained arterial sections of femoral arteries. A greater neointimal formation **(arrows)** can be observed in the untreated **(A)** than in the atorvastatin treated rabbit **(B). M,** medial layer (magnification ×100). **Bottom,** The atorvastatin-treated group had less severe stenoses estimated both as intima/media ratio **(solid bars)** or as a percentage of the luminal area occupied by the lesion **(open bars).** Data are the mean ± SD of seven treated and nine untreated rabbits. *p = 0.001 vs. untreated; **p = 0.038 vs. untreated.

layer and in areas of the neointima without macrophages, suggesting that VSMC could be responsible for its synthesis. The diminution in the area occupied by MCP-1 in the neointima can not be explained solely by the overall reduction in the lesion size, since MCP-1 decreased by 90% while the lesion was only reduced by 50%–60%.

NF-κB activation in the lesions. NF-κB activation was studied by Southwestern histochemistry to determine the presence and localization of activated NF-κB in situ. The amount of stained nuclei in the tissue was calculated according to a

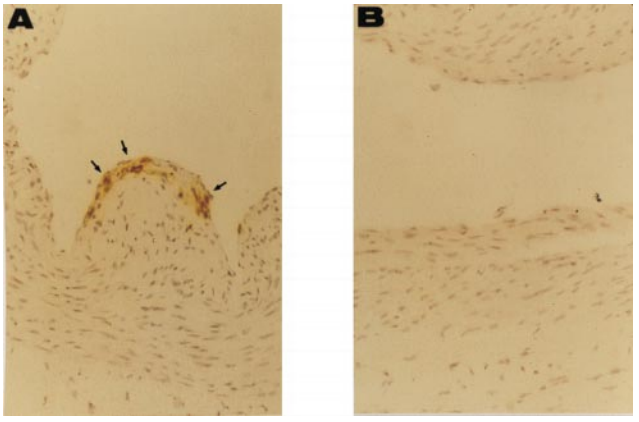


Figure 3. Macrophage detection by immunohistochemistry in arterial sections. Photomicrographs of arterial sections stained with RAM11 antibody, specific for rabbit macrophages. A representative section of each treatment group is shown. **A,** Nontreated group. **B,** Atorvastatin-treated group. None of the control animals showed any stain (not shown). **Arrow,** presence of macrophages **(brown)** (magnification ×400).

semiquantitative score ranging from 0 to 5. We found NF-κB activity in the neointima and media in four out of seven animals studied in the untreated group (all ≥2), while only in two out of seven in the atorvastatin treated (only one >2) and in none of the controls. Atorvastatin treatment induced a mean reduction of 60% in the neointimal activation and 65% in the media. The binding of the labeled probe was specific since no staining was found when the mutant NF-κB oligonucleotide was used in untreated animals (Fig. 5). By double staining with the digoxigenin-labeled oligonucleotide and an antimacrophages and anti-alpha-actin antibodies, we found that both cell types were activated in the lesions (Fig. 5E and F, respectively). However, due to the larger amount of VSMC, it seems that their contribution is much greater than that of macrophages.

NF-κB activation in the aorta and liver. Since atherogenic diet by itself has been shown to induce NF-κB activation in the liver (12), we also studied by gel shift assay whether this factor was activated in the liver and aorta. NF-κB was more activated in the liver (Fig. 6, top) and aorta (bottom) of untreated animals than of the atorvastatin group. Competition assays with cold NF-κB, but not with an unrelated cold oligonucleotide (AP-1), abolished the appearance of the labeled complex, indicating the specificity of the interaction. We also studied the activation of another transcription factor (AP-1), involved mainly in the regulation of extracellular matrix and metalloproteinase genes, in the liver and aorta of the rabbits. AP-1 activity was increased in both organs of untreated rabbits, but the treatment with atorvastatin did not induce any change in its activation (Fig. 6). The assay was specific, as demonstrated by the competition of the binding with cold AP-1, but not with cold NF-κB.

Modulation of NF-κB activation and MCP-1 expression by atorvastatin in VSMC. To establish whether the observed atorvastatin effect on MCP-1 and NF-κB could be, at least in part, due to a direct effect of the drug on the VSMC, we

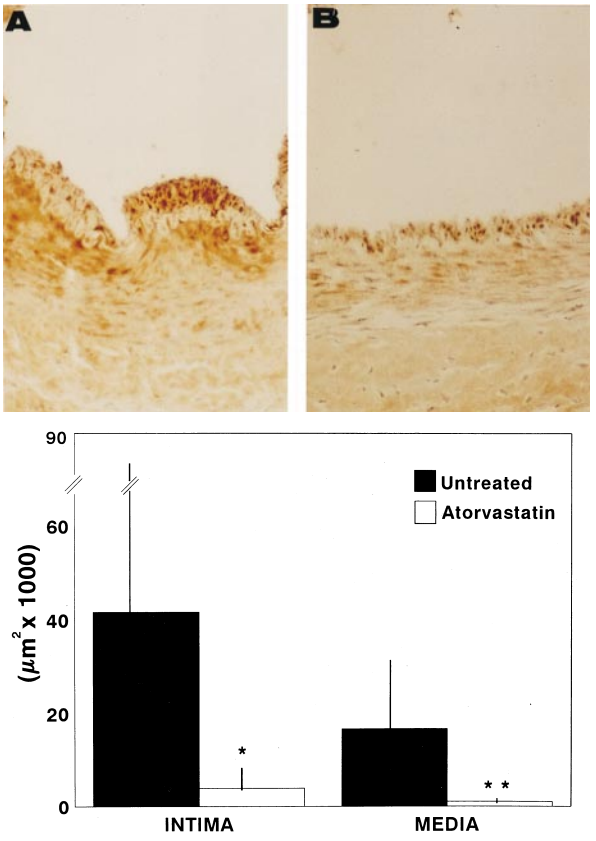


Figure 4. MCP-1 detection by immunohistochemistry in arterial sections. **Top,** Photomicrographs of arterial sections stained with an anti–MCP-1 antibody. Both the neointima and the media showed staining for MCP-1. A representative section of an untreated animal is shown in **A** and of an atorvastatin treated rabbit in **B.** Control arteries showed no staining for MCP-1 (not shown) (magnification ×400). **Bottom,** Closed bars represent the mean area stained for MCP-1 in the untreated animals and open bars the mean area stained in treated animals. Data are the mean ± SD of seven treated and nine untreated rabbits. *$p < 0.01$ vs. untreated and **$p < 0.05$.

studied the expression and activation of these factors induced by tumor necrosis factor alpha (TNFα) in VSMC. TNFα (100 U/mL) induced a sevenfold increase of MCP-1 mRNA. Preincubation of the cells with 10^{-7} mol/L atorvastatin for 1 h reduced the MCP-1 expression (Fig. 7). NF-κB activation was also induced by 100 U/mL TNFα (7.5-fold; $p < 0.03$) and the preincubation of the cells with 10^{-7} mol/L atorvastatin for 1 h significantly diminished this activation (60%, $p < 0.05$).

Discussion

In the present work we have demonstrated that in a rabbit model of atherosclerosis the-HMG-CoA-reductase inhibitor atorvastatin reduces the inflammatory component in the atherosclerotic lesion. This effect could help to explain the reduction in ischemic events observed upon statin treatment in clinical trials.

Inflammation and lesion stabilization. It is well demonstrated that statins reduce the size and the extent of the lesions. This is, however, not a convincing explanation for the reduction of the acute ischemic events that they achieve, since it is well known that these events are usually due to the rupture of unstable plaques independently of their size (4). In our model, treatment with atorvastatin resulted in less severe lesions, but also in the abolition of macrophage infiltration and in the reduction of MCP-1. It must be emphasized that atorvastatin treatment was not started at the beginning of the experiment but after 4 weeks of hyperlipemic diet, in an attempt to imitate the conditions of human atherosclerosis, where statins are used in most cases once atherogenesis has begun.

Most thromboses of vessels with atherosclerotic lesions are preceded by a fissure that allows the lipid core to contact the circulating blood (13,14). An inflammatory reaction plays an important role in this rupture, and the presence of macrophages in the surroundings of the fissured plaques from patients with acute coronary syndromes has also been demonstrated (4,5). These cells can release proteolytic enzymes that degrade the fibrotic cap of the lesion, making it prone to rupture (15). The presence of macrophages in the lesion is favored by chemoattractants such as oxidized low density lipoprotein (ox-LDL) and MCP-1. In this sense, the lipid-lowering effect of atorvastatin would not only reduce the generation of ox-LDL but also the synthesis of MCP-1. This chemokine is present in human atherosclerotic lesions, mainly in the areas infiltrated by macrophages (6,16).

In spite of the absence of macrophages in the lesions of atorvastatin-treated rabbits, a certain amount of MCP-1 was expressed, suggesting that the drug may modulate additional mechanisms involved in the neointima infiltration, such as the expression of adhesion molecules and other chemokines. In this sense, it is noteworthy that the genes of many chemokines, adhesion molecules and other inflammation-related proteins possess NF-κB recognition sites in their promoters (17), and thus the reduction of NF-κB activation achieved with the treatment could be a pivotal point controlling different parameters related to inflammation within the plaque.

Mechanisms of the reduction of vascular inflammation by atorvastatin. MCP-1 reduction by the treatment seemed to be related to a diminution in NF-κB activity, and this effect seems to be specific on NF-κB since the activation of AP-1, another transcription factor commonly overexpressed during inflammation and also related to vascular injury (18), was not modified. NF-κB is responsible for the induction of MCP-1 transcription after stimulation by lipopolysaccharide (LPS), interleukin 1-beta, TNFα and phorbol esters (7). It has been recently shown that Lovastatin possesses a certain antioxidant action (19), and it would therefore be interesting to study whether this effect is related to the inhibition of NF-κB activity.

NF-κB activity was greater in the aorta and liver of untreated animals, that is, in tissues exposed only to hypercholesterolemia without local injury, suggesting a role for circulating cholesterol. This is in accordance with the results of Liao et al. (20) and provides a link between hyperlipidemia and plaque rupture. However, we cannot exclude the possibility that atorvastatin also had a direct effect on the atherosclerotic

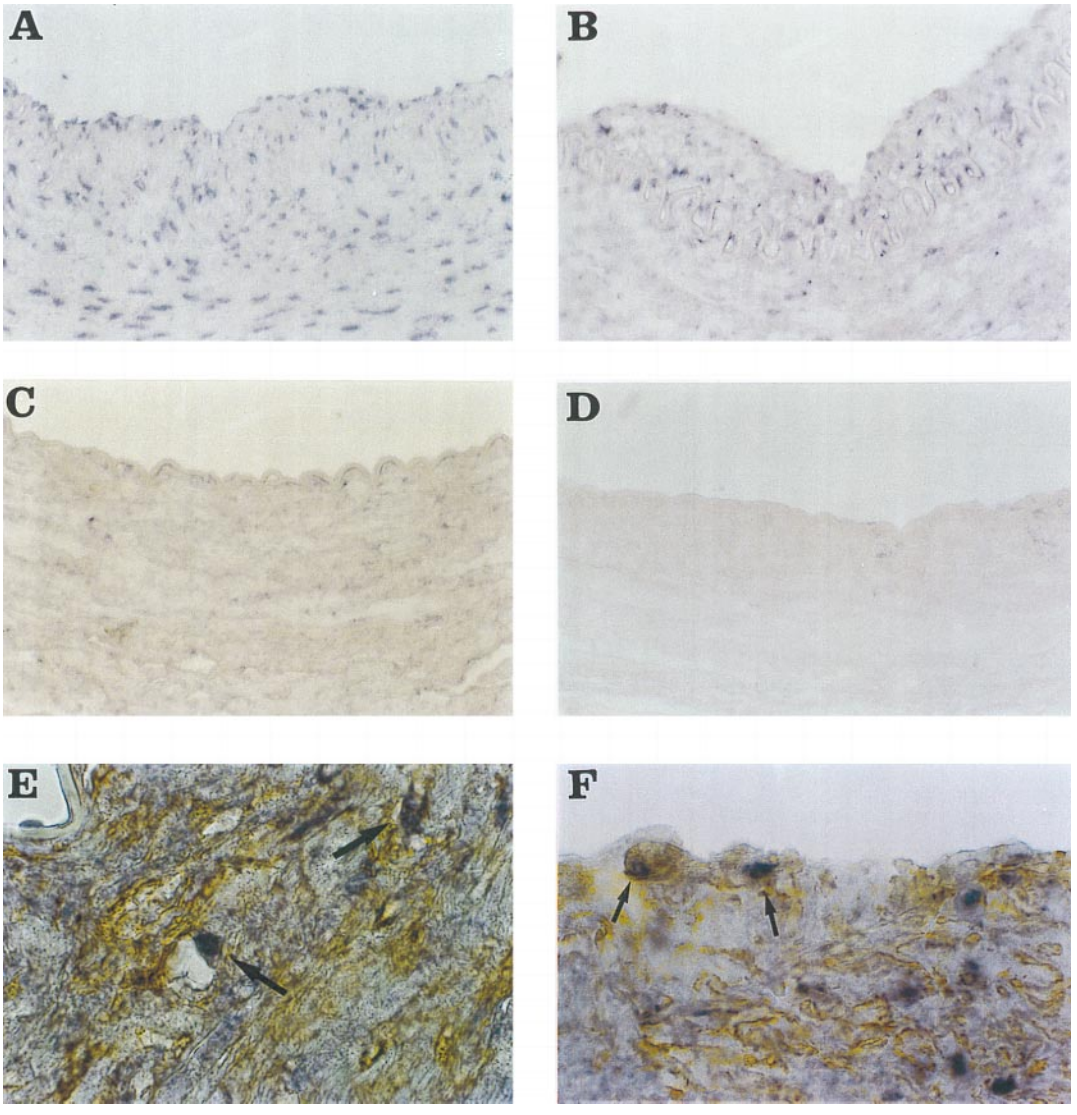


Figure 5. NF-κB activity in the lesions. NF-κB activity was determined by Southwestern histochemistry in untreated **(A)**, atorvastatin treated **(B)** and control **(C)** animals. The binding of the labeled probe is specific since no staining is found with the mutant oligonucleotide **(D)** (magnification ×400). A detail of the localization of the NF-κB activity by double staining with anti–macrophage antibody is shown in **E** and with anti-alpha-actin in **F** (magnification ×1000). The arrows indicate the presence of a nuclei stained for NF-κB **(blue)** and surrounded by a cytoplasm **(brown)** stained with the antibody specific for macrophages **(E)** and for VSMC **(F)**.

lesions, since the HMG-CoA reductase is present in every cell as an integral enzyme of the cholesterol synthesis route (21). Because to this, other authors have shown that lovastatin reduces neointimal formation in a model of normocholesterolemic rabbits (22). To test this hypothesis, we carried out in vitro experiments on atorvastatin effect on MCP-1 expression and NF-κB activation induced in VSMC by TNFα, which is usually present during vascular injury (23), thus excluding the possibility of actions mediated by cholesterol reduction. In these experiments we found that atorvastatin reduced both MCP-1 expression and NF-κB activity, suggesting that part of the in vivo effects of atorvastatin could be due to a direct effect of the drug on the atherosclerotic plaques. However, the present study was not designed to investigate the importance of the direct effect of atorvastatin on the vessel wall. Furthermore, clinical evidence has shown consistently that lowering cholesterol reduces the incidence of acute ischemic events not only when achieved by statins, but also when other cholesterol-lowering drugs (24) or even nonpharmacological approaches are used (25). Thus, it seems wise to speculate that cholesterol reduction was probably the main responsible of the effects seen in our in vivo experiment, although a direct action of atorvastatin could also play a role in these effects.

Study limitations. This study has been carried out in an experimental model of accelerated atherosclerosis that differs from the chronic lesions observed in human disease. However, our findings strongly agree with observations made in human atherosclerosis in the sense that statins reduce the incidence of

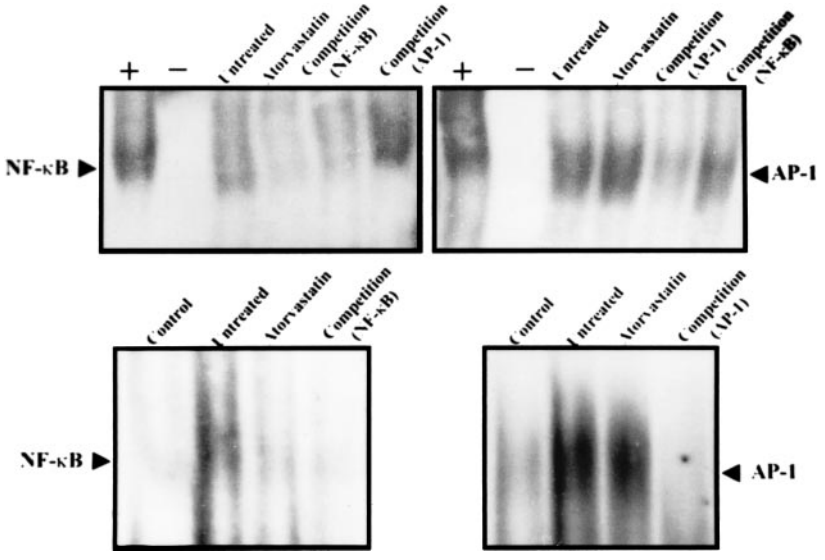
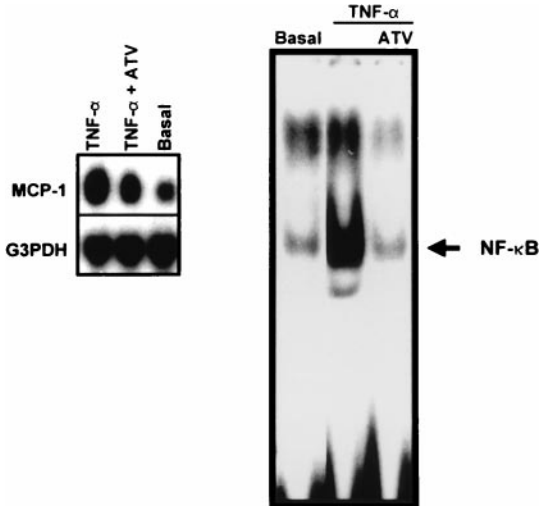


Figure 6. NF-κB and AP-1 activity in the aorta and liver of rabbits. The activation of NF-κB **(left)** and AP-1 **(right)** was determined in the aorta **(bottom)** and liver **(top)** of the rabbits by electrophoretic mobility shift assay (EMSA). Cellular extracts obtained from the aorta and liver of the animals from the same group were pooled. (+) positive control with Hela nuclear extracts and (−) negative control without cellular extract. Representative of two experiments done.

acute coronary syndromes and that inflammation plays a role in the pathophysiology of these syndromes. Finally, it must be noticed that we have not tested the incidence of plaque rupture in the animals, but only the presence of cells and mediators related to inflammation. Unfortunately, there is no animal model in which spontaneous plaque rupture occurs. Nevertheless, the strong relation found in humans between inflammatory cells and plaque thrombosis supports the use of macrophage infiltration as a marker of plaque unstability.

Conclusions. As a whole, our results demonstrate that atorvastatin reduces the inflammatory features in the neointima and suggest a potential explanation for the reduction of ischemic events observed with HMG-CoA-reductase inhibitors in clinical trials. They also indicate that a part of this effect could be due to a direct action of this drug on the atherosclerotic lesions, although further work is needed to evaluate its contribution.

Figure 7. Atorvastatin modulation of NF-κB activity and MCP-1 expression in VSMC. Vascular smooth muscle cells were growth-arrested by serum deprivation for 48 h and then preincubated with 10^{-7} mol/L atorvastatin for 1 h before the addition of 100 U/mL of TNFα. Cells were then cultured for 6 h to study MCP-1 expression by Northern blot and for 1 h to determine NF-κB activity by EMSA. G3PDH mRNA expression was used to normalize the MCP-1 expression. A representative blot of three experiments done is shown.



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