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Cardiovascular Research 57 (2003) 168–177

Cardiovascular Research

www.elsevier.com/locate/cardiores

Simvastatin reduces NF-κB activity in peripheral mononuclear and in plaque cells of rabbit atheroma more markedly than lipid lowering diet

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Received 28 May 2002; accepted 26 July 2002

Abstract

Objective: To study whether simvastatin reduces inflammation in atherosclerosis beyond its hypolipidemic effects. **Methods:** Twenty-four rabbits with induced femoral injury and on an atherogenic diet were randomized to normolipidemic diet ($n=9$), or to continue the atherogenic diet while receiving simvastatin 5 mg/kg/day ($n=9$) or no treatment ($n=6$) for 4 weeks. **Results:** As compared with no treatment, the normolipidemic diet significantly reduced lipid levels, while simvastatin produced nonsignificant reductions. In spite of this, NF-κB binding activity in peripheral mononuclear cells was reduced in the simvastatin group [2,958±5,123 arbitrary units (a.u.)] as compared with no treatment (49,267±20,084 a.u.; $P<0.05$) and normolipidemic groups (41,492±15,876 a.u.; $P<0.05$) (electrophoretic mobility shift assay). NF-κB activity in the atherosclerotic lesions was also reduced by simvastatin as compared to nontreated animals (4,108±3,264 vs. 8,696±2,305 nuclei/mm^2; $P<0.05$), while the normolipidemic diet induced only a nonsignificant diminution ($P>0.05$) (Southwestern histochemistry). Similarly, simvastatin decreased macrophage infiltration (4.6±12 vs. 19±12% of area staining positive; $P<0.05$) and the expression of interleukin-8 (24±12 vs. 63±21%; $P<0.05$) and metalloproteinase-3 (16±3 vs. 42±28%; $P<0.05$) (immunohistochemistry), while the reduction achieved by normolipidemic diet in all these parameters was again nonsignificant ($P>0.05$). **Conclusions:** These findings suggest that simvastatin reduces inflammation in atherosclerotic plaques and in blood mononuclear cells more than expected for the lipid reduction achieved.

Keywords: Atherosclerosis; Cholesterol; Gene expression; Infection/inflammation; Macrophages; Statins

1. Introduction

Inflammation plays a role in atherosclerotic plaque thrombosis [1,2]. Mononuclear cells enter the atherosclerotic lesions where they differentiate into macrophages which, along with other inflammatory cells, release enzymes that degrade matrix proteins, weakening the fibrous cap and making plaques prone to rupture and subsequent thrombosis [3–8]. Lipid reduction, by either dietary manipulation or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) treatment, has

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Time for primary review 24 days.

0008-6363/02/$ – see front matter
PII: S0008-6363(02)00619-3

been shown to reduce inflammation in experimental models of atherosclerosis [8–10].

Statins do also seem to have direct beneficial effects on atherosclerotic lesions beyond cholesterol reduction. Several works have demonstrated that these drugs decrease the extent of atherosclerosis in experimental models without hyperlipidemia and in the absence of lipid reduction [11–13]. Also, in in vitro studies, statins improve nitric oxide production [14,15], reduce proliferation and migration of vascular smooth muscle cells [16], superoxide formation [17], and tissue factor activity [18].

In the present study we have explored the ability of simvastatin to directly reduce inflammation in a rabbit model of atherosclerosis. In order to assure that a part of the antiinflammatory effect was nonlipid-mediated, the animals treated with simvastatin were kept on a hypercholesterolemic diet, thus partially balancing the hypolipidemic effect of the drug. The results were compared with those of another group undergoing greater lipid reduction by diet manipulation in the absence of statin treatment. To study inflammation, the activity of nuclear factor-κB (NF-κB) was assessed in peripheral blood mononuclear cells (PBMCs) and in atherosclerotic lesions. This is a transcription factor which enhances the expression of several proinflammatory molecules [19,20], and that is activated in several inflammatory processes, including atherosclerosis [21–25]. Furthermore, in the atherosclerotic lesions we assessed several inflammatory features like the infiltration of macrophages, the expression of matrix metalloproteinases (MMPs) -1, -2 and -3, and of chemokines such as interleukin-8 (IL-8) and interferon-inducible protein-10, whose genes contain NF-κB sites in their promoters [19,20].

2. Methods

2.1. Experimental model

2.1.1. Induction of atherosclerosis

Twenty-four New Zealand male rabbits weighing 3.5–4 kg were housed in individual cages, quarantined for 7 days before use, and treated according to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health. An atherogenic diet (2% cholesterol and 6% peanut oil, Letica, Barcelona, Spain) was started on day 0. One week later, vascular injury was induced in the femoral arteries using desiccated nitrogen gas according to a previously described technique with minimal modifications [26]. Animals were anesthesized with 5 mg/kg xylazine (Rompun, Bayer AG, Leverkusen, Germany) and 35 mg/kg ketamine (Ketolar, Parke-Davis, Ann Arbor, MI, USA). Using sterile technique and cefazoline antibiotic cover (125 mg/kg, Llorente Laboratories, Madrid, Spain), segments of both femoral arteries (1–2 cm in length, and 1 cm below the inguinal ligament) were isolated between airtight ligatures. Local spasm was prevented by the topical administration of 2% lidocaine (Braun, Barcelona, Spain). The isolated femoral artery segments were cannulated with a 27-gauge needle, a puncture was made in the other end for escape, and blood was removed by a saline flush. Endothelial damage was induced by the passage of industrial nitrogen at a rate of 80 ml/min for 8 min. After air-drying, the ligatures were removed, hemostasis was achieved by local pressure, and the wound was closed with a 4.0 vicryl subcuticular suture. One week after surgery, the animals were randomized to receive 5 mg/kg/day of simvastatin ($n=9$) or no treatment ($n=6$) while kept on atherogenic diet. A third group was randomized to normolipidemic diet ($n=9$) in order to achieve a greater lipid reduction than the simvastatin group. After 4 weeks, the animals were sacrificed. The dose of simvastatin was chosen on the basis of previous experiments designed to rule out toxicity and any effect of the drug on chow consumption. It is important to remember that an extrapolation to human beings cannot be done, as rabbits need higher doses of statins than patients to obtain similar reduction in blood lipids. The weight of the animals was controlled weekly to adjust the dose of drug.

Four animals fed standard chow without any experimental intervention were used as healthy controls.

2.1.2. Harvesting of damaged vessels

At the time of killing, the rabbits were anesthetized and the femoral arteries were exposed. Then, the animals were killed with an overdose of pentobarbital (Abbot, Madrid, Spain) and the femoral arteries were cannulated, fixed in situ with 4% buffered formaldehyde at 100 mmHg pressure, and removed. The arteries were kept for 24 h in 4% buffered formaldehyde and afterwards in 70% ethanol until paraffin embedded.

2.1.3. Blood lipid measurements

Rabbits were bled from a marginal ear vein for determination of blood lipids. Plasma samples were collected 24 h post-meal at the start of the study and at weeks 2, 4, and 6. Total cholesterol, chylomicrons, low-density lipoproteins (LDLs), high-density lipoproteins (HDLs), very low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), and triglycerides were measured by standard enzymatic assays (Sigma Chemical Co., St. Louis, MO, USA).

2.2. Studies in atherosclerotic lesions

Every artery was divided in four equal fragments previously to embedding it in paraffin. Then, all fragments underwent cross-sectioning into 4-μm thick pieces.

2.2.1. Morphometry

Multiple sections of every fragment, chosen at regular

intervals, were hematoxylin-stained, and were qualitatively analysed to identify the zone with the most severe stenosis. Once located, that section was chosen for morphometric analysis, and the adjacent sections were used for immuno- and Southwestern histochemistry. Morphometry was performed using the Olympus semiautomatic analysis system with Micro Image™ software (version 1.0 for windows). Preparations were digitized via an Olympus microscope (BH-2) at 400 magnification, connected to a CCD videocamera.

2.2.2. Immunohistochemistry

Macrophages were identified with a monoclonal anti-rabbit macrophage antibody (RAM11, Dako, Denmark) according to a previously described technique [27]. IL-8 and interferon protein-10 were detected with polyclonal goat anti-human IL-8 and interferon-inducible protein-10 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). MMP-1, MMP-2 and MMP-3 proteins were immunolocalized by using monoclonal mouse anti-human MMP-1, MMP-2, and MMP-3 antibodies (Oncogene Research Products, Cambridge, UK). MMP-3 is easily detected in this way, probably because it binds to glycosamynoglicans in the tissue. RAM11 (84 μg/ml) was applied for 1 h, and anti IL-8 (5 μg/ml), interferon-inducible protein-10 (10 μg/ml), MMP-1, MMP-2 and MMP-3 (5 μg/ml) antibodies were applied overnight. As secondary antibodies, a biotin-labeled donkey anti-goat IgG (Amersham Iberica S.A., Spain) was used for IL-8 and interferon-inducible protein-10, and a biotin-labeled goat anti-mouse IgG (Dako) for RAM11, MMP-1, MMP-2 and MMP-3. Secondary antibodies (1:200) were applied for 30 min and the ABComplex/HRP (Dako) was then added for another 30-min period. The sections were stained for 10 min at room temperature with 3,3′-diaminobenzidine (DAB, Dako). Finally, sections were counterstained with hematoxylin and mounted in Pertex (Medite, Germany). In each experiment, negative controls without the primary antibody or using a nonrelated antibody were included to check for nonspecific staining. Images were analyzed with the same equipment used for morphometric analysis. They were preprocessed with the color segmentation tool, which allows to separate the positive objects from the rest of the image. Results were expressed as percentage of immunostained area.

2.2.3. Southwestern histochemistry

The distribution and DNA-binding activity of NF-κB in situ was detected by this method, described in our laboratory [28], using a digoxigenin-labeled double-stranded DNA probe with a specific consensus sequence that binds to NF-κB (5′-AGTTGAGGGGACTTTCCCAGGC-3′). Preparations without probe were used as negative controls, and a mutant NF-κB probe was used to test the specificity of the technique (5′-AGTTGAGGCTCCTTTCCCAGGC-3′). Image analysis was performed using the same methods as for immunohistochemistry and the results were expressed as positive nuclei/mm^2. Of interest, hematoxylin–eosin staining is not performed with this technique. Then, violet staining indicates only nuclei with activated NF-κB.

For colocalization studies, immunohistochemistry for vascular smooth muscle cells (HHF-35, monoclonal anti-α actin, Sigma, Spain) and macrophages (RAM-11) were carried out on slides directly from the final wash of Southwestern histochemistry protocol without drying.

2.3. Studies in peripheral mononuclear cells

2.3.1. Isolation of peripheral blood mononuclear cells

At weeks 2, 4 and 6, 30 ml of blood were drawn from the ear vein for PBMC isolation. Blood samples were diluted in phosphate-buffered saline (PBS) 1:1 and the cells were separated in a 10 ml ficoll gradient (lymphocyte isolation solution, Rafer, Spain) by centrifugation at 2000 *g* for 30 min. PBMCs were collected, washed twice with cold PBS and resuspended in buffer A for later processing [26].

2.3.2. Electrophoretic mobility shift assay (EMSA)

Protein extracts pooled from mononuclear cells were prepared as previously described [29]. Protein concentration was quantified by the BCA method (Pierce, Rockford, IL, USA). NF-κB consensus oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′) was ^{32}P-end-labeled with 10 U of T4 polynucleotide kinase (Promega, Madison, WI, USA). NF-κB composition was assessed with antibodies against the p65 and p50 subunits (Santa Cruz Biotechnology) and incubated for 1 h. To test the specificity of the assay, a 100-fold excess of unlabeled probe was added to the binding reaction.

2.4. Statistical analysis

Statistical analysis was performed with GraphPAD InStat (GraphPAD Software). Data are presented as mean±S.D. and were analyzed using the multiple comparison Kruskal–Wallis test followed by a Dunn's test. Differences were considered to be significant when $P<0.05$ (two-tailed).

3. Results

3.1. Lipid levels

At the moment of randomization (week 2), the atherogenic diet induced a marked increase in all lipid parameters, except in HDL levels (Table 1). At the end of the study (week 6), animals randomized to normolipidemic diet showed significantly lower levels of total cholesterol, VLDL, IDL, LDL, and triglycerides than those randomized to no treatment, without significant changes in HDL levels.

Table 1
Lipid values (mg/dl)

	Day 0	2 Weeks	6 Weeks		
			NT	ND	SV
TC	71±23	2,092±548	4,140±479	453±258*‡	2,065±1,020
VLDL	10±8	833±328	2,006±422	218±184*	1,003±454
TG	93±29	178±94	201±66	67±22†‡	167±59
IDL	10±6	263±93	438±175	97±57 *‡	300±104
LDL	63±32	305±133	314±96	134±54†	254±96
HDL	35±9	40±9	44±9	30±10	39±9

NT=No treatment (hyperlipidemic diet alone); ND=normolipidemic diet; SV=simvastatin plus hyperlipidemic diet; TC=total cholesterol; VLDL=very low-density lipoprotein; TG=triglyceride; IDL=intermediate-density lipoprotein; LDL=low-density lipoprotein; HDL=high-density lipoprotein; *$P<$ 0.001 and †$P<0.01$ vs. NT; ‡$P<0.05$ vs. SV.

Moreover, they had also significantly lower total cholesterol, IDL and triglyceride levels than those switched to simvastatin. The simvastatin group showed a marked decrease in blood lipid levels with respect to the non-treated group, though it did not reach statistical significance.

3.2. Morphometry

Maximal lesion size of femoral arteries of the animals randomized to normolipidemic diet was lower than those randomized to no treatment (0.104 ± 0.149 vs. 0.650 ± 0.688 mm^2; $P<0.05$). The reduction observed in the simvastatin group did not achieve statistical significance (0.166 ± 0.297 mm^2; $P>0.05$).

3.3. NF-κB activity in peripheral blood mononuclear cells

To establish whether the high lipid levels obtained in this model could induce NF-κB activation in blood mononuclear cells, we studied this transcription factor along the time. At weeks 2, 4, and 6, peripheral blood mononuclear cells from four to six hyperlipidemic nontreated animals (depending on the week) were obtained, and nuclear extracts were pooled. All determinations were done with 10 μg of total protein from each pool, and experiments were done in duplicate. As depicted in Fig. 1A, NF-κB activity increased during these periods of time (1-, 3.6- and 6-fold, respectively). The main components of NF-κB activity were p50 and p65 subunits as evidenced in supershift assays using antibodies against them.

At week 6, NF-κB activity was reduced in the simvastatin group [2,958±5,123 arbitrary units (a.u.)] as compared with no treatment (49,267±20,084 a.u.; $P<0.05$) and normolipidemic groups (41,492±15,876 a.u.; $P<0.05$) (Fig. 1B). There were no significant differences between these last groups ($P>0.05$). These results were confirmed by analysis of pooled nuclei extracts of peripheral blood mononuclear cells of five animals from each group (experi-

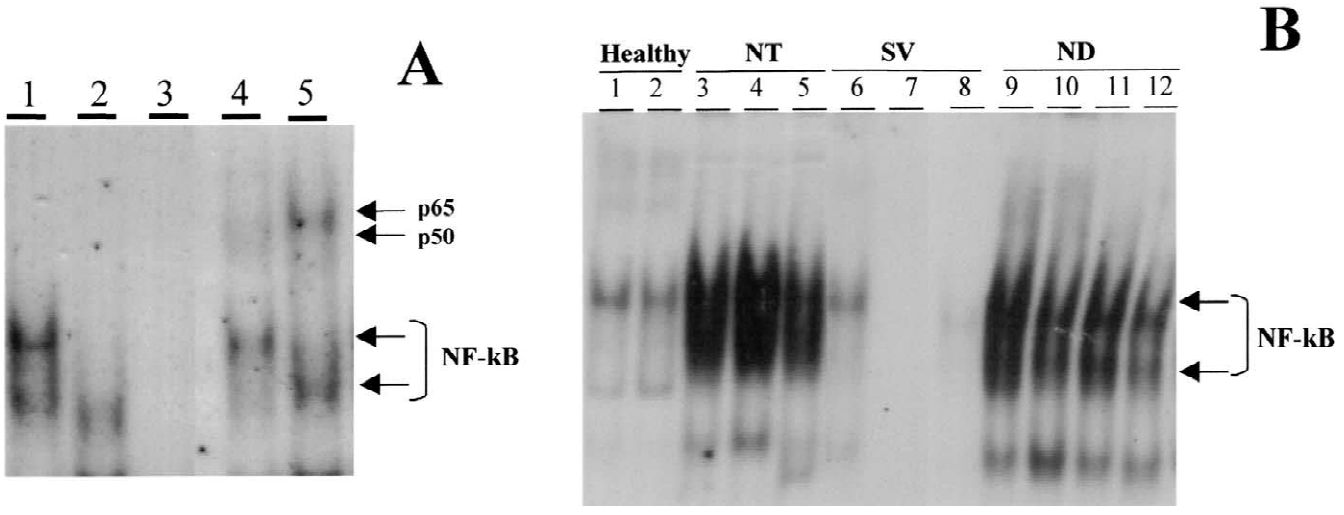

Fig. 1. NF-κB activity in PBMCs. (A) NF-κB activity was assayed to study the effect of the increase in serum lipid levels on PBMC activation. Nuclear extracts of three to five different nontreated hyperlipidemic animals at each time period were pooled and incubated with the NF-κB probe. Representative gel shows animals from weeks 6 (lane 1), 4 (lane 2), and 2 (lane 3). Lanes 4 and 5 represent superhifts using antibodies for subunits p50 and p65 of NF-κB, respectively, in animals from week 6. (B) Representative examples of NF-κB activity in PBMCs of different treated groups (EMSA). Healthy animals display very low activity, which is greatly increased in atherosclerotic non-treated rabbits (NT). As compared with NT animals, normolipidemic diet (ND) induced a mild decrease in NF-κB activity, which was more marked in rabbits treated with simvastatin (SV).

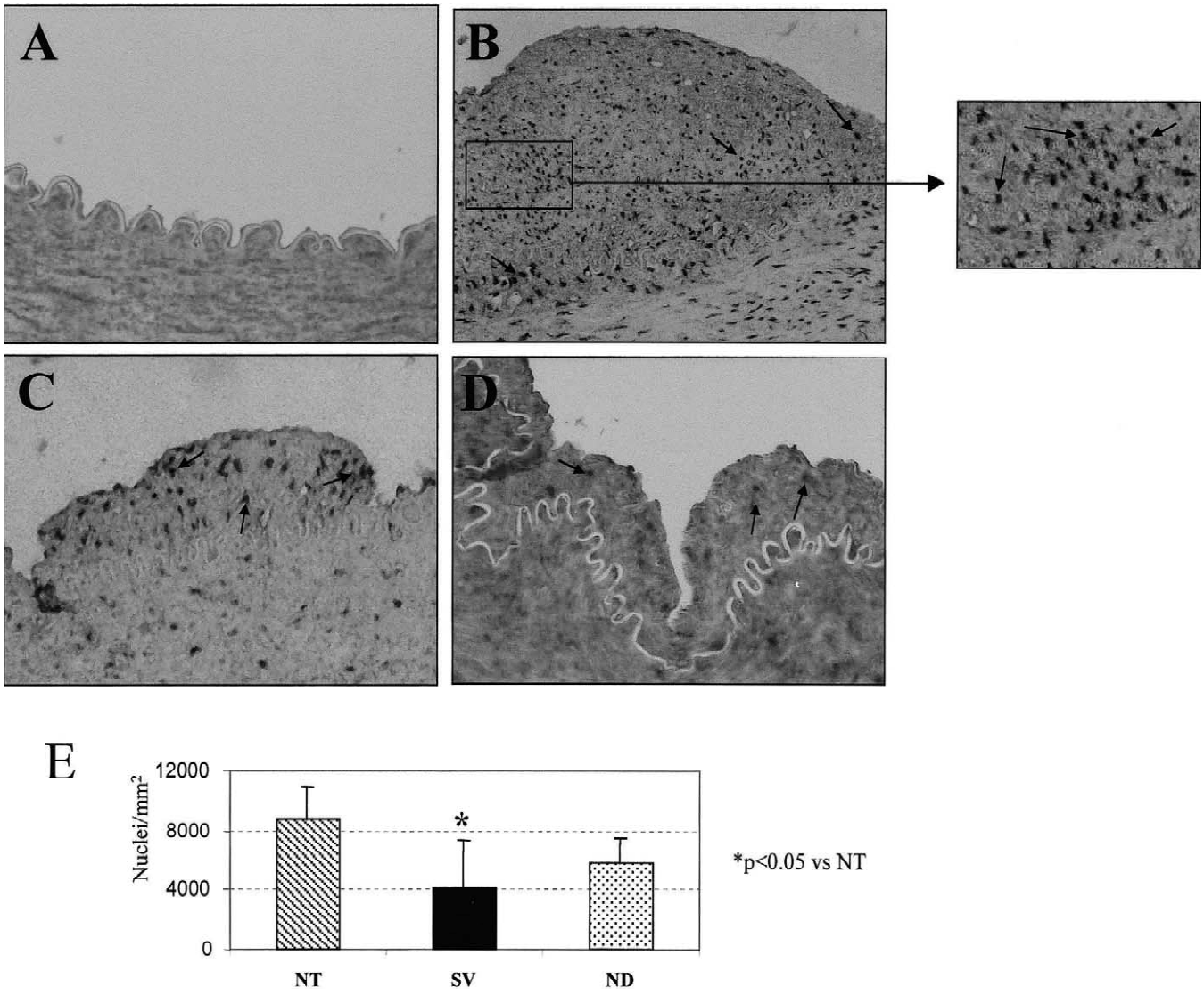

Fig. 2. Arterial NF-κB activation determined by Southwestern histochemistry. Arterial sections were hybridized with an oligonucleotide containing the consensus sequence of the NF-κB recognition site. (A) Healthy, (B) nontreated, (C) normolipidemic diet and (D) simvastatin-treated animals. Arrows remark positive nuclei staining in violet. Magnification ×400 and ×1000 (detail). Bar graph shows the quantification of positive nuclei per mm^2 in the lesions. *P* values refer to comparisons with NT animals. See text for details.

ments were done in duplicate). In pool analysis, NF-κB binding activity in PBMCs from nontreated rabbits increased sixfold as compared to healthy animals. Simvastatin and normolipidemic diets reduced NF-κB activity by 90 and 23%, respectively.

3.4. NF-κB activity in the atherosclerotic lesions

NF-κB activity was also studied in the atherosclerotic lesions by Southwestern histochemistry. This technique allows the localization of activated nuclear factors in the cellular nucleus.

As shown in Fig. 2, a marked NF-κB activity was found in the neointima of nontreated hyperlipidemic animals in relation to healthy animals, while it was reduced in the simvastatin group (4,108±3,264 vs. 8,696±2,305 nuclei/mm^2, $P<0.05$). However, despite a greater reduction in blood lipids, the diminution showed in rabbits switched to normolipidemic diet was not statistically significant (5,778±1,684 nuclei/mm^2; $P>0.05$).

Colocalizations performed by the combination of Southwestern histochemistry with immunohistochemistry for macrophages and vascular smooth muscle cells showed that both cell types displayed NF-κB activity in all the treatment groups (Fig. 3).

3.5. Macrophage infiltration and chemokine expression in the atherosclerotic lesions

In relation to nontreated hyperlipidemic animals, the simvastatin group showed a significant diminution of the percentage of neointima staining positive for macrophages

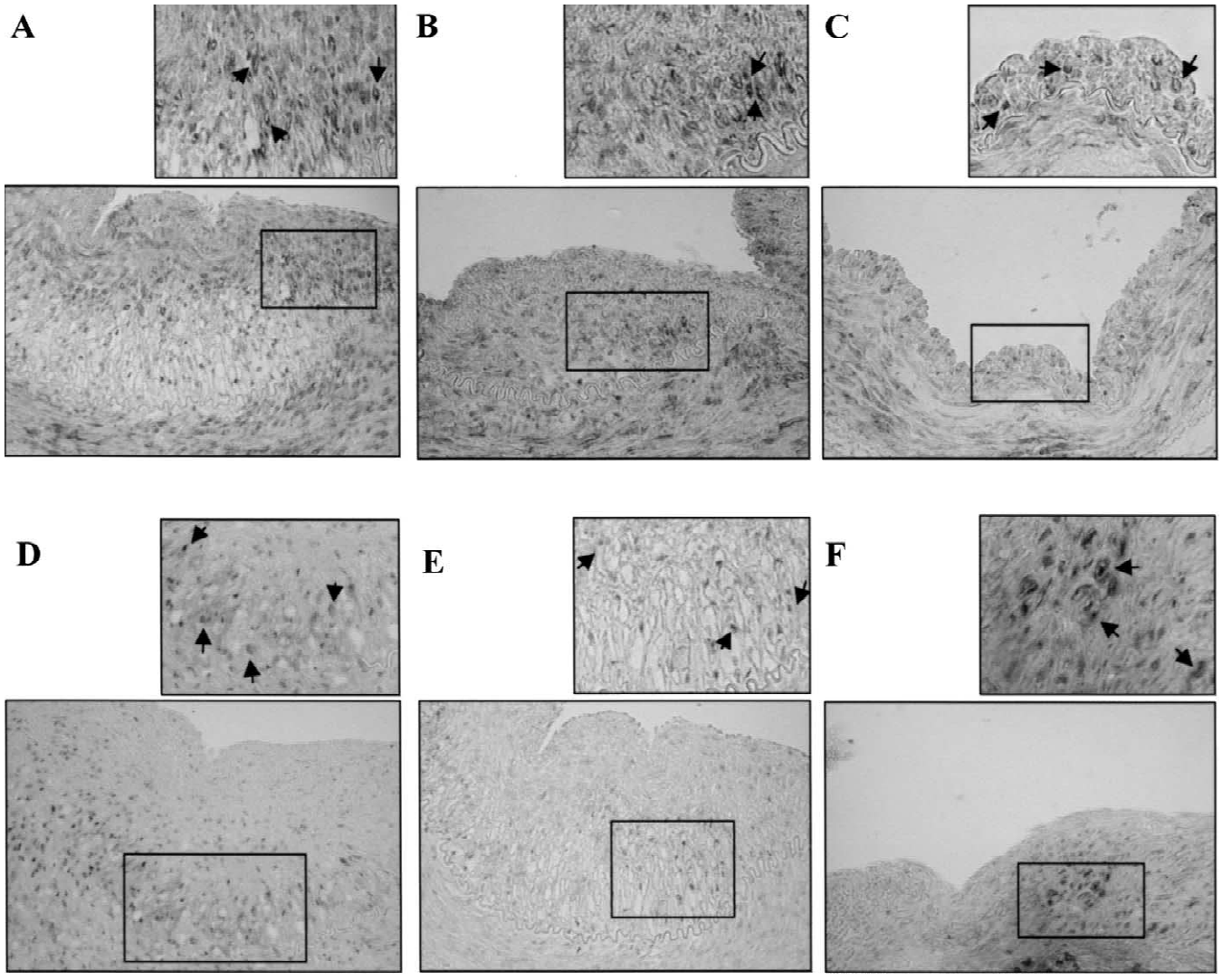

Fig. 3. Colocalizations for vascular smooth muscle cells and macrophages with NF-κB activity. Upper: colocalization for vascular smooth muscle cells (HHF-35) and NF-κB activity (Southwestern histochemistry). Lower: colocalization for macrophages (RAM 11) and NF-κB activity. A and D: Non-treated animals; B and E: normolipidemic diet; C and F: animals treated with simvastatin. Nuclei positive for active NF-κB stain in violet. Positivity for vascular smooth muscle cells and macrophages is displayed in brown. Smooth muscle cells are located peripherally, while macrophages are visible mainly in the center of the lesions. Both cell types display NF-κB activity in all treatment groups (arrows). Magnification ×400.

(19±12 vs. 4.6±12%; $P<0.05$). The decrease presented by animals randomised to normolipidemic diet did not reach statistical significance (5.5±6%; $P>0.05$) (Fig. 4, upper). In addition, simvastatin-treated animals displayed a significant reduction of neointimal staining for IL-8 with respect to nontreated animals (24±12 vs. 63±21%; $P<0.05$). This reduction was milder and not significant in the group switched to normolipidemic diet (30±23%; $P>0.05$) (Fig. 4, middle).

Positive staining for interferon-inducible protein-10 was present in the 50±21% of the neointima surface in nontreated hyperlipidemic animals. The normolipidemic diet and simvastatin groups showed a marked diminution of positive staining, though it was not statistically significant (35±22 and 34±22%, respectively; $P=0.35$ for multiple comparison test). Healthy animals did not show positive staining for macrophages, IL-8, and interferon-inducible protein-10 (not shown).

3.6. Metalloproteinase expression in the atherosclerotic lesions

There were no significant differences among the three groups in the percentage of neointima staining positive for MMP-1 (nontreated hyperlipidemic: 55±7%, normolipidemic diet: 41±18%, simvastatin: 38±29%; $P=0.39$) and MMP-2 (nontreated hyperlipidemic: 32±21%, normolipidemic diet: 16±19% and simvastatin: 14±20%; $P=0.14$). By contrast, MMP-3 staining was significantly decreased in simvastatin group with respect to nontreated hyperlipidemic animals (16±3 vs. 42±28%; $P<0.05$), while the rabbits randomized to normolipidemic diet

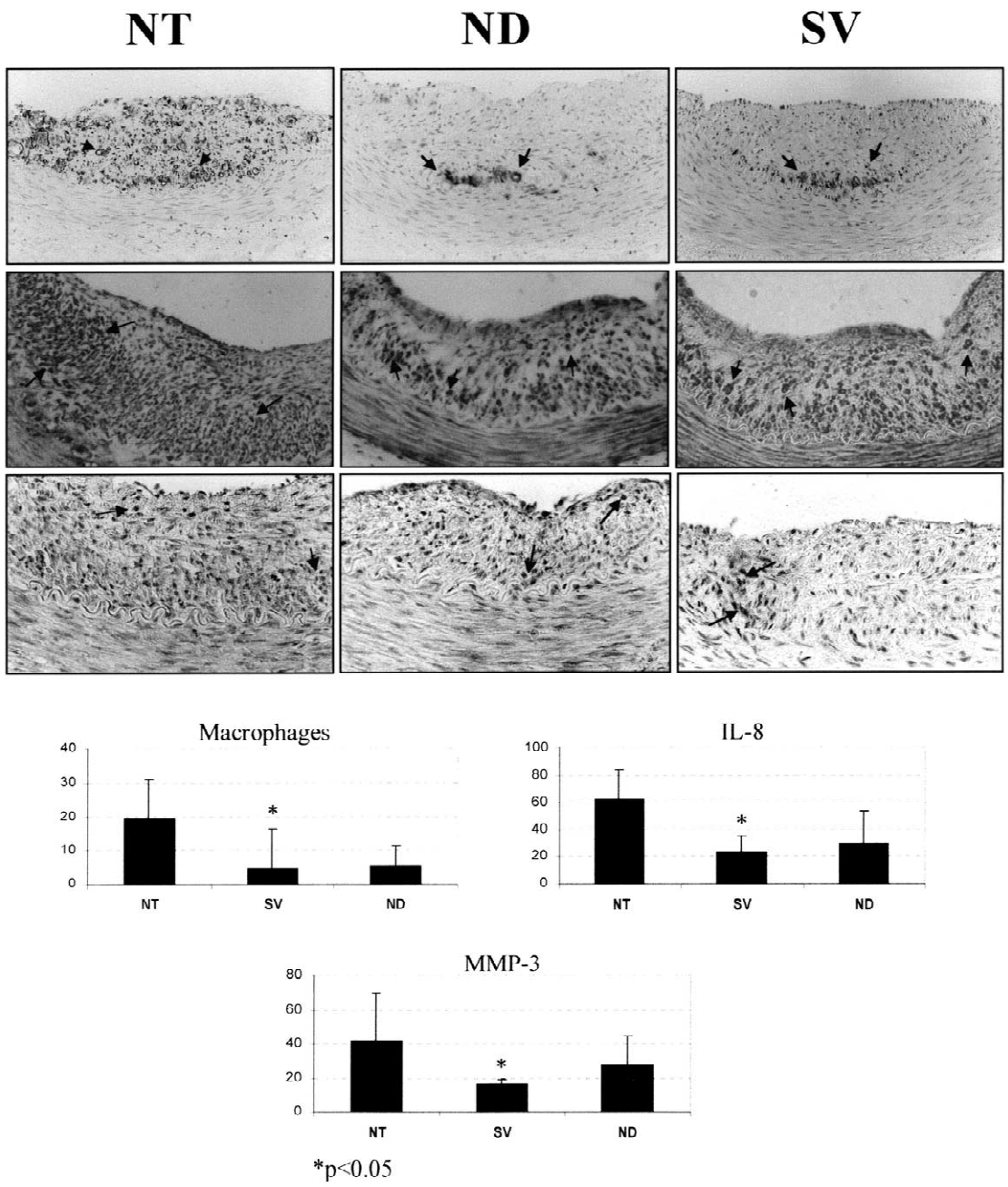

Fig. 4. Macrophage, IL-8 and MMP-3 detection by immunohistochemistry. Representative examples of femoral artery sections stained with anti-macrophage (RAM11) (upper), anti IL-8 (middle) and anti-MMP-3 (lower) antibodies. NT: Nontreated animals; ND: normolipidemic diet and SV: simvastatin-treated rabbits (magnification ×400). Quantifications are displayed in the bar graphs on the right. *P* values refer to comparisons with NT animals. See text for details.

presented a nonsignificant diminution (28±17%; $P>0.05$) (Fig. 4, lower). Differences between simvastatin and normolipidemic groups were nonsignificant. Healthy animals did not show positive staining for MMPs (not shown).

4. Discussion

4.1. Effect of statins on atherosclerosis

Statins decrease the incidence of acute coronary events [30–32], which are due to plaque thrombosis. Accordingly, they have been demonstrated to improve endothelial function [33] and to reduce inflammation [9,10] and blood thrombogenicity [34]. In addition, these drugs have lipid-independent effects on atherosclerosis. They reduce neointimal formation in normocholesterolemic rabbits, even in the absence of changes in lipid levels [11–13], and blunt the decrease in constitutive nitric oxide synthase expression induced by oxidized LDLs in cultured vascular endothelial cells, under conditions independent of extracellular lipid levels [14,15].

4.2. Effect of simvastatin on NF-κB activation

In a rabbit model of atherosclerosis, we further investigated a possible direct effect of HMG-CoA reductase inhibition on the inflammatory activity. For this purpose, after induction of atherosclerosis by vascular damage and hyperlipidemic diet, we randomized the animals to a normolipidemic diet or to keep the hyperlipidemic diet while receiving simvastatin, or no treatment. Animals switched to normolipidemic diet had, at the end of the study, a significant decrease in total cholesterol, LDL, VLDL, IDL and triglycerides, when compared to non-treated animals, and in total cholesterol, IDL and triglycerides when compared to those switched to simvastatin. As expected, given the high blood lipid levels achieved with the experimental diet employed in our study, the reduction of lipid levels by simvastatin was milder than that of animals treated with the normolipidemic diet.

Despite the absence of a significant decrease in lipid levels, animals treated with simvastatin showed a greater reduction of NF-κB activity in circulating mononuclear cells and in the atherosclerotic lesions than those randomized to normolipidemic diet. NF-κB activity in the lesions was due to both macrophages and smooth muscle cells. At least in part, the decrease in NF-κB activity was due to some lipid-independent mechanism. In this regard, statins block the enzyme HMG-CoA reductase at the beginning of the mevalonate pathway, lowering cholesterol synthesis and depleting cells of mevalonate and its isoprenoid derivatives [35], and therefore resulting in a reduction of the intracellular pool of isoprenylated proteins. Isoprenylation is necessary for the activation of small G-proteins, implicated in signal transduction from several receptors [36] that activate transcription via NF-κB [37]. In cultured vascular smooth muscle cells and mononuclear cells, we have shown previously that the addition of farnesylpyrophosphate and geranylgeranylpyrophosphate (necessary for protein isoprenylation) reversed the decrease in NF-κB activity induced by HMG-CoA reductase inhibition [38]. Then, the decrease in NF-κB activity found in the present work in animals treated with simvastatin could be due, at least in part, to the reduction of isoprenylated G-proteins.

4.3. Effect of simvastatin on macrophage infiltration and chemokine and MMP expression in the atherosclerotic lesions

In the lesions, we explored the effect of the different treatments on macrophage infiltration and on the expression of MMP-1, -2, and -3, which have been previously demonstrated to be present near these cells in atherosclerotic plaques [39–41]. We also examined the expression of IL-8 and IP-10, two chemokines involved in the recruitment of monocytes and lymphocytes into the vascular wall [42,43].

There was a significant diminution of macrophage infiltration, and IL-8 and MMP-3 expression in the atherosclerotic lesions of simvastatin-treated animals, while those randomized to normolipidemic diet presented moderate, but non-significant, reductions. Although the need to use a multiple comparison test was partially responsible for this lack of significance, the decrease achieved by normolipidemic diet in these parameters was milder. This suggests a stronger anti-inflammatory effect of the statin compared to that obtained by diet lipid lowering. The decrease in macrophage infiltration and the subsequent lowering of MMP-3 expression may, in part, be due to the reduction of IL-8 expression. The diminution of NF-κB activity seems to be the hallmark of these findings, since it controls the expression of IL-8 and other molecules involved in monocyte recruitment [19,26,44], as well as that of MMP-3 [20].

Williams et al. [45] showed a reduction of macrophage infiltration in atherosclerotic monkeys after treatment with pravastatin and diet manipulation to avoid changes in serum lipid levels, but no other inflammatory features were evaluated. Also, more recently, simvastatin has been found to reduce inflammation in a model of carrageenan-induced foot pad edema in mice [11], and to decrease leukocyte rolling and adherence in rats [46] and apolipoprotein E-deficient mice [47] beyond its lipid-lowering action. The present results are in agreement with these works and further strengthen the concept of the antiinflammatory effects of statins.

4.4. Limitations

The findings reported here have been obtained in an experimental model of early accelerated atherosclerosis, that differs from the chronic lesions of human disease in several aspects as, for example, the fact that vascular smooth muscle cell proliferation determines for a great deal lesion formation. Also, the dose of simvastatin is higher than that used in the clinical practice. However, it must be emphasized that the hyperlipidemia obtained by diet manipulation was also greater than that usually seen in human beings, although it is of the same range to that seen in other experimental models [24,26]. Moreover, the fact that statins reduce the incidence of acute coronary events and the observation that inflammation is related to plaque instability in humans fit with the effects of simvastatin described in this study and make them very probably to be responsible in part for the benefits of these drugs in the clinical practice.

5. Conclusions

This study shows that the HMG-CoA reductase inhibitor simvastatin has an anti-inflammatory effect on experimental atherosclerosis beyond its ability to reduce cholesterol

levels. This effect is also evident in circulating blood mononuclear cells, whose inflammatory activity correlates with that of atherosclerotic plaques. This work reinforces the idea of a direct beneficial action of statins on atherosclerosis. However, clinical studies are needed to determine whether this effect is also present in humans at the doses used in clinical practice.

Acknowledgements

This work was supported by grants from Fundación Ramón Areces, Fundación Española del Corazón, Fondo de Investigación Sanitaria (98/1269), and Merck Sharp and Dohme Spain, Madrid, Spain.

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