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ORIGINAL ARTICLE

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The protective role of atorvastatin on function, structure and ultrastructure in the aorta of dyslipidemic rabbits

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Abstract Responses to both an endothelium-dependent (acetylcholine 10^{-9}–10^{-5} mol/l) and an endothelium-independent vasodilator (sodium nitroprusside 10^{-10}–10^{-6} mol/l) were studied in aortic rings from rabbits fed or not with a diet containing 0.5% cholesterol plus 14% coconut oil for 14 weeks and treated or not with atorvastatin (2.5 mg/kg/day)**.** Morphometric and ultrastructure analyses were also performed. Rabbits fed the dyslipidemic diet presented higher plasma cholesterol and triglyceride concentrations than controls ($P<0.05$). This was associated with intima–media thickening and, consequently, aortic stenosis (29±3%) since vessel cross-sectional area did not change. Endothelial cells presented numerous alterations in dyslipidemic rabbits. Atorvastatin treatment only reduced plasma levels in dyslipidemic rabbits ($P<0.05$), which were nevertheless higher than those of controls. In addition, it prevented the reduction in acetylcholine relaxation in hypercholesterolemic animals. Atorvastatin administration also enhanced the response to acetylcholine in rabbits fed a control diet. Likewise, atorvastatin treatment reduced lesion area and consequently increased aortic lumen in dyslipidemic rabbits but did not modify media thickening. It also prevented the majority of the ultrastructural changes observed in endothelial cells. In conclusion, chronic atorvastatin treatment exerts a protective role in vascular function, structure and ultrastructure even in the presence of high cholesterol and triglyceride plasma levels.

Keywords Dyslipidemia · Endothelial function · Statin · Ultrastructure · Vascular wall

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Introduction

Serum cholesterol level elevation is the triggering event in the development of atherosclerosis, which is the major cause of morbidity and mortality in the Western population due to its cardiac and cerebral complications [26]. Even before morphological changes occur, lipid accumulation in the blood vessel wall during hypercholesterolemia produces functional endothelial alterations [41], characterized by an impaired endothelium-dependent vasodilatation [15, 23, 28, 45]. Intimal thickening, mainly as a consequence of macrophage-derived foam cell accumulation, is the main feature of atherosclerotic lesion, although smooth muscle cells also contribute to the lesion through cell accumulation and extracellular matrix production [8, 33]. Changes in endothelial and smooth muscle cell ultrastructure have also been associated with atherosclerosis [5, 6].

Statins are lipid-lowering agents that reduce cholesterol synthesis by inhibiting the 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis. Inhibition of HMG-CoA reductase not only reduces cholesterol synthesis, but also induces an upregulation of cell surface low-density lipoprotein (LDL) receptors, thus leading to a reduction in circulating levels of LDL–cholesterol [9]. Clinical trials have demonstrated that statins are effective in both primary and secondary prevention of coronary heart disease [36, 37]. This effect seems to be proportional to the achieved reduction in cholesterol. In ad-

dition, administration of statins is associated with amelioration of endothelial dysfunction [3, 12, 44] and reduction of the atherosclerotic lesion [4, 12, 14, 18, 38]. However, whether these effects depend on the normalisation of cholesterol levels is not yet well established. Moreover, there is no information available regarding statin effects on endothelial and smooth muscle cell ultrastructure. Therefore, the aim of this study was to evaluate the effect of atorvastatin on functional, structural and ultrastructural changes in aorta from rabbits with dyslipidemia.

Materials and methods

Animals

Experiments were conducted in 32 male New Zealand rabbits (Granja Cunicular San Bernardo, Navarra, Spain) initially weighting 2088±35 g, maintained under controlled light and temperature conditions. The animals were fed either a normal rabbit chow or a diet containing 0.5% cholesterol plus 14% coconut oil (Mucedola s.r.l., Milan, Italy) for 14 weeks and had free access to tap water. During the same period, half of the animals from each diet group were treated with atorvastatin (2.5 mg/kg per day) given in the food. At the end of this period, blood samples were collected in prechilled glass tubes containing ethylenediamine tetra-acetic acid (EDTA) at a final concentration of 10^{-7} mol/l through a catheter inserted in the ear artery of awake rabbits. Plasma triglyceride and cholesterol levels were measured using colorimetric reactions employing commercial kits (Boehringer-Mannheim, Mannheim, Germany). After taking blood samples, the animals were anesthetized with sodium pentobarbital (25 mg/kg, iv). The descending thoracic aorta was exposed through a midline incision, excised and the proximal aorta cut into two pieces for structural and functional studies. All experimental procedures were approved by the animal care and use committee of Universidad Complutense, according to the guidelines for ethical care of experimental animals of the European Community.

Aortic ring preparation

One of the pieces of proximal aorta was immediately transferred to ice-cold Kreb' s bicarbonate buffer (composition in mmol/l: NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11), was cleaned of periadventitial tissue and was cut transversally into ring segments (3.0 mm in length).

Each ring was placed inside a 5-ml heated tissue bath filled with Krebs's bicarbonate buffer (37°C) bubbled with 95% O_2–5% CO_2 and was suspended between two L-shaped stainless steel hooks. The upper one was attached to a force-displacement transducer (model FT03, Grass) and coupled to a computerized system (McLab model 8E, AD Instruments) for measurement of isometric tension. Rings were allowed to equilibrate for 60–90 min with changes of buffer every 15 min and with several adjustments of length until the baseline tension stabilized at 2 g. In preliminary experiments, we found that 2 g of resting tension is optimal for the expression of KCl-induced contraction of rings of aorta obtained from normal rabbits. When the isometric tension was stable, the experiments were initiated by obtaining a reference contractile response to KCl (80 mmol/l). At the end of the experiment, the presence or absence of functional endothelium was verified in all phenylephrine-contracted aortic rings by observing whether or not relaxation occurred upon exposure to acetylcholine (10^{-5} mol/l).

The vasorelaxing response to the endothelium-dependent vasodilator, acetylcholine (10^{-9}–10^{-5} mol/l) was studied in aortic rings from normo- and dyslipidemic rabbits precontracted with a submaximal dose of phenylephrine (10^{-6} mol/l). The relaxing response to the nitric oxide (NO) donor sodium nitroprusside (10^{-10}–10^{-6} mol/l) was also evaluated. The degree of preconstriction of rings was similar among all groups.

Histology and morphometric analysis

Aortic segments were fixed in 15% sodium phosphate-buffered formaldehyde, processed, impregnated and embedded in paraffin and cut into 3–4-µm sections using a microtome. The sections were stained with hematoxylin–eosin, Masson trichrome and orceine. Morphometric (quantitative) determination of the area of the intima, media and the vessel was performed with a MICROM image analyser (Hardware IMCO 10, Kontron Bildanalyse, Software Microm IP, Microm, Spain) as previously described [43]. Briefly, all microscopic images of the sections were recorded on videotape with a videocamera and the histological sections were digitalized, segmented–colored and traced for calculation of the areas. To determine the luminal area, the cross-sectional area enclosed by the internal elastic lamina was corrected to a circle applying the form factor l×2/4 to the measurement of the internal elastic lamina, where *l* is the length of the lamina. Vessel area was determined by the cross-sectional area enclosed by the external elastic lamina corrected to a circle applying the same form factor (l×2/4) to the measurement of the external elastic lamina. Number of elastic layers and the media thickness were measured in orceine-stained sections using a QWIN Leica image analyzer (Leica Imaging Systems, Ltd, Cambridge, England). Measurements in five different places along the vessel were done in each section choosing the narrowest and the widest points, with the other three being randomly chosen between the two.

Ultrastructure analysis

Aortic samples were fixed in 2.5% glutaraldehyde in phosphate buffer. After rinsing, the specimens were post-fixed in 4% osmium tetroxide in phosphate buffer at 4°C, and dehydrated in graded acetone. The fixed samples were included in araldyte and contrasted with uranyl acetate and lead citrate, as previously described [43]. Smooth muscle cell size was determined by their width as measured through the nucleus. At least 20 cells in both internal and external media layers were measured in each animal.

Drugs

Atorvastatin was obtained from Parke Davis SL (Barcelona, Spain). Drugs for functional studies were purchased from Sigma Chemical Co., St. Louis, Mo. All the products for histological and ultrastructural analysis were obtained from Merck (Darmstadt, Germany). Stock solutions for in vitro studies were prepared in distilled water and diluted to desired concentrations with buffer immediately before the experiment. Concentrations are expressed as final molar concentration in the organ chamber.

Calculations and statistical analysis

The relaxing response of phenylephrine-preconstricted aortic rings is expressed as percent reduction of tension in preconstricted state. Results are expressed as mean±SEM of rings from 7 rabbits, unless otherwise specified. Vascular reactivity dose-response curves were compared using multivariate analysis of variance for repeated measures (MANOVA) using the Complete Statistical System program (CSS, Statoft Inc.,Tulsa, Okla.). All other data were analysed using a one-way analysis of variance, followed by a Newman-Keuls test if differences were noted. The null hypothesis was rejected when the *P* value was less than 0.05.

Results

The intake of a diet enriched with cholesterol and coconut oil induced an increase ($P<0.05$) in cholesterol and triglyceride plasma levels as compared with animals fed a control diet (55.3±1.7 mmol/l versus 1.2±0.2 mmol/l and 6.9±0.7 mmol/l versus 1.1±0.2 mmol/l, respectively). Atorvastatin administration (2.5 mg/kg/day) significantly ($P<0.05$) attenuated this increase (38.2±1.3 mmol/l and 3.9±0.7 mmol/l, respectively). By contrast, atorvastatin did not modify either cholesterol or triglyceride levels in rabbits fed a control diet. At the end of the experiment, no differences were observed in body weight increase among any group.

The relaxing response to acetylcholine was blunted ($P<0.05$) in aortic rings from rabbits fed a dyslipidemic diet compared with rings from control rabbits (Fig. 1). Atorvastatin administration was able to prevent a reduction in the relaxation response to acetylcholine induced by the hypercholesterolemic diet (Fig. 1). Likewise, atorvastatin was also able to increase acetylcholine-induced relaxation in rabbits fed a control diet (Fig. 1). By contrast, neither the diet nor atorvastatin treatment was able to modify the sodium nitroprusside-induced vasorelaxation (data not shown).

Dyslipidemic rabbits clearly showed the presence of vascular alterations as compared with animals fed a control diet. As shown in Table 1, the lumen area was reduced in aortas from dyslipidemic rabbits. In fact, 29±3% of the area encompassed by the internal elastic lamina were occupied by atherosclerotic lesion (Fig. 2A). In addition, the media cross-sectional area was larger in aortas from dyslipidemic rabbits than in aortas from control animals, although no changes were observed in vessel size (Table 1). Atorvastatin treatment reduced the size of the lesion (Table 1), degree of stenosis (4±1%) and consequent lumen increase in vessels from dyslipidemic rabbits (Fig. 2B). All these changes took place without modifying the media cross-sectional area (Table 1). The administration of atorvastatin in animals fed a control diet had no effect on vascular structure.

Aorta ultrastructure revealed important abnormalities in comparison with the artery wall of control rabbits. Intimal thickening was largely a consequence not only of lipid-laden monocyte-derived macrophages (foam cells), but also of smooth muscle cell accumulation and numerous collagen fibers (Fig. 3A). Smooth muscle cells presented ovoid shape and abundant rough endoplasmatic reticulum and prominent Golgi apparatus, suggesting a synthetic state. In addition, they contained many lipid inclusions (Fig. 3A). In fact, some foam cells were derived from smooth muscle cells. Likewise, there were pools of extracellular lipid (cholesterol crystals and numerous liposomes), and abundant necrotic foam cells found in the subintimal area (Fig. 3A), although only a few T-cells were present. Endothelial integrity was disrupted in

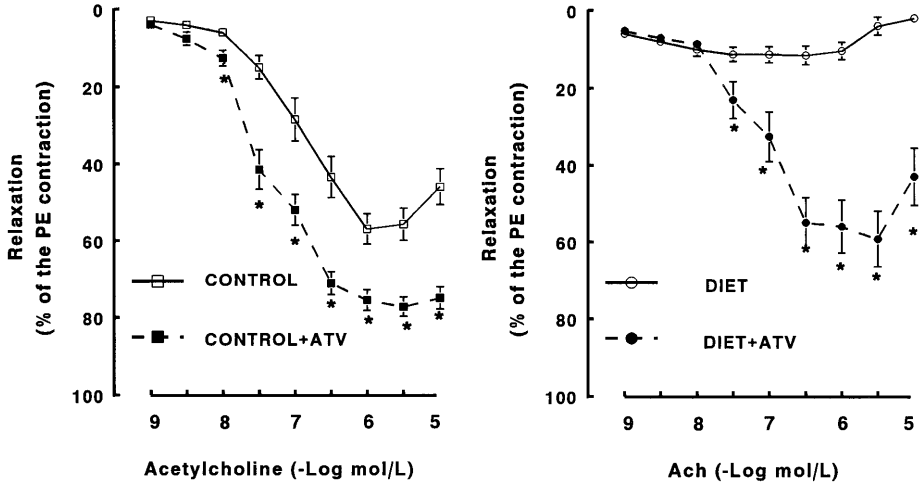


Fig. 1 Line graph illustrating the vasorelaxations induced by acetylcholine (10^{-9}–10^{-5} mol/l) in aortic rings precontracted with a submaximal dose of phenylephrine (10^{-6} mol/l) from rabbits fed for 14 weeks either a diet containing 0.5% cholesterol+14% coconut oil (*right panel*) or a control diet *(left panel*), treated (*full symbols*) or not (*empty symbols*) with atorvastatin (2.5 mg/kg/day). Values are mean±SEM of rings from 7 rabbits. **P*<0.05 compared with rings from untreated rabbits

Table 1 Aortic lumen, media cross-section, vessel cross-section and lesion area of rabbits fed either a diet containing 0.5% cholesterol+14% coconut oil or a control diet, treated or not with atorvastatin (2.5 mg/kg per day) for 14 weeks. Values are mean±SEM of 6–7 rabbits

Group	Aortic lumen (mm^2)	Media cross-section) (mm^2	Vessel cross-section (mm^2)	Lesion area (mm^2)
Control	2.48±0.10	0.57±0.06	3.09±0.16	–
Diet	1.76±0.15*	0.78±0.06*	3.33±0.52	0.68±0.20*
Control+atorvastatin	2.47±0.13	0.52±0.02	2.99±0.13	–
Diet+atorvastatin	2.39±0.08**	0.81±0.02*	3.29±0.13	0.20±0.08*,**

**P*<0.05 compared with control rabbits
***P*<0.05 compared with atorvastatin-untreated rabbits fed a diet containing 0.5% cholesterol+14% coconut oil for the same period of time

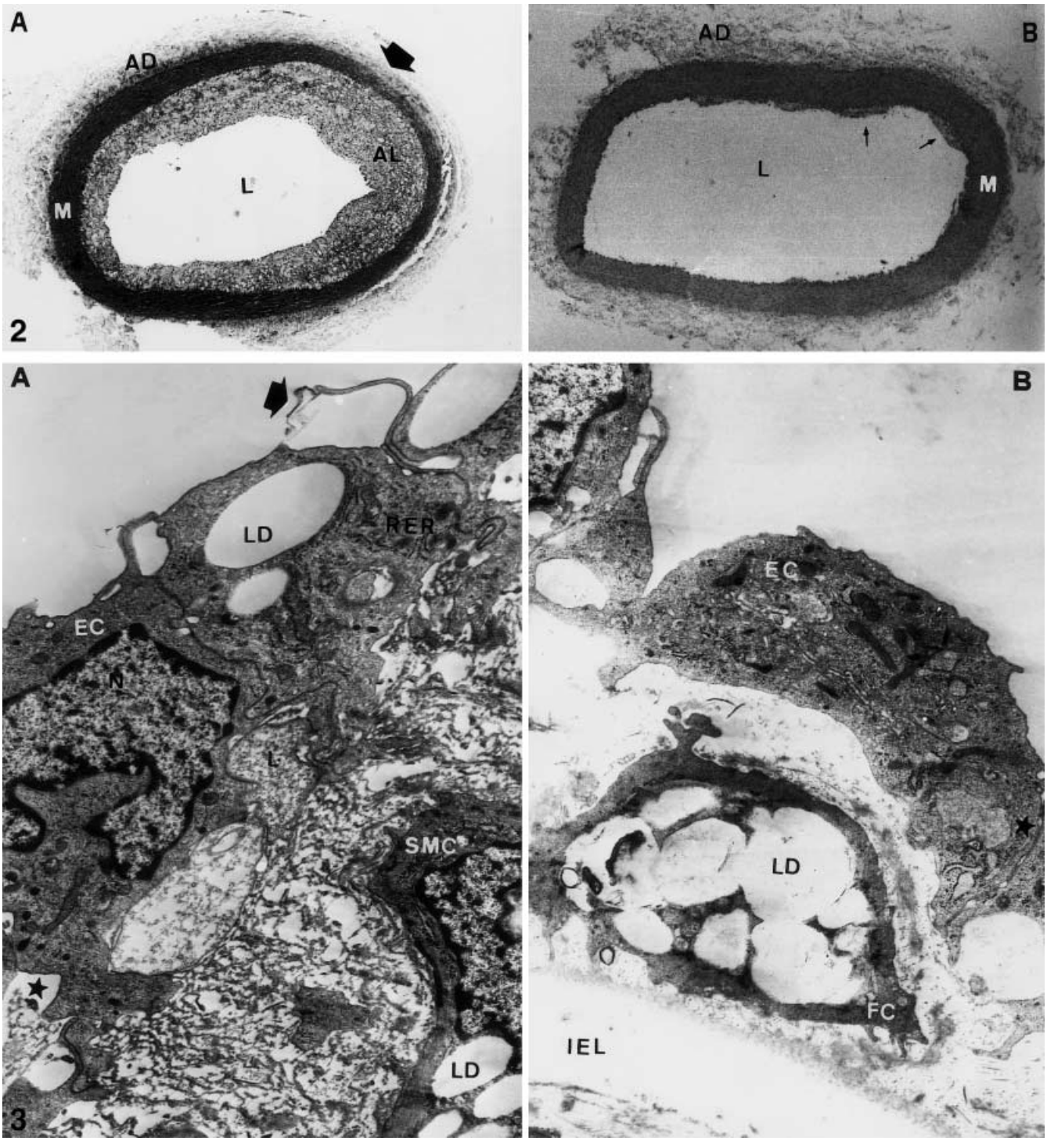


Fig. 2 **A** Masson tricrome-stained section of aorta from untreated rabbits fed a diet containing 0.5% cholesterol+14% coconut oil showing a circumferencial atherosclerotic lesion (*AL*), which produced an important narrowing of lumen aorta (*L*). Media layer (*M*) shows a large thickening variability. **B** Hematoxylin and eosin-stained section of aorta from atorvastatin-treated rabbits fed a diet containing 0.5% cholesterol+14% coconut oil. Atorvastatin treatment significantly reduced lesion area (*arrows*) and thickening variability media (*M*). Original magnification ×34. *AD* adventitia

Fig. 3 Thin-section electron micrographs demonstrating a typical atherosclerotic lesion from untreated (**A**) or atorvastatin-treated (**B**) rabbits fed a diet containing 0.5% cholesterol+14% coconut oil**.** In untreated animals, endothelial cells (*EC*) are rounded with polygonal nuclei (*N*) presenting numerous lipid droplets (*LD*) and a prominent rough endoplasmatic reticulum (*RER*) and liposomes (*L*). They also presented big gaps in the intercellular junctions (*asterisk*). Broken digitate (*arrow*). Smooth muscle cells (*SMC*) with numerous lipid droplets are also present in the atherosclerotic lesion. In atorvastatin-treated rabbits, a more elongated EC shape approaches that of the control group. Gaps are minimal in the intercellular junctions (*asterisk*). Atherosclerotic lesion size is reduced, although some cells are present. Original magnification ×4800. *IEL* internal elastic layer; *FC* foam cell

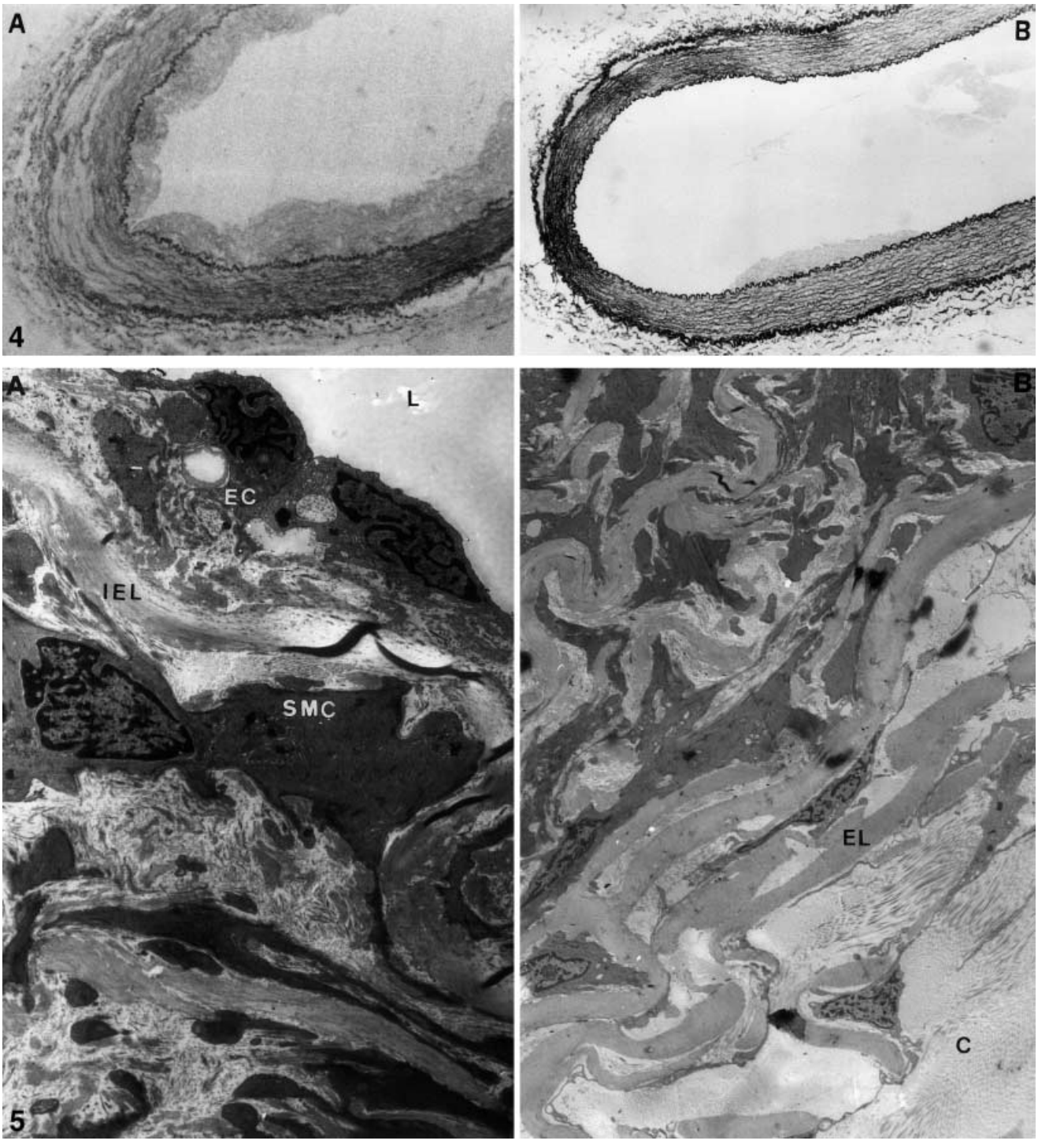
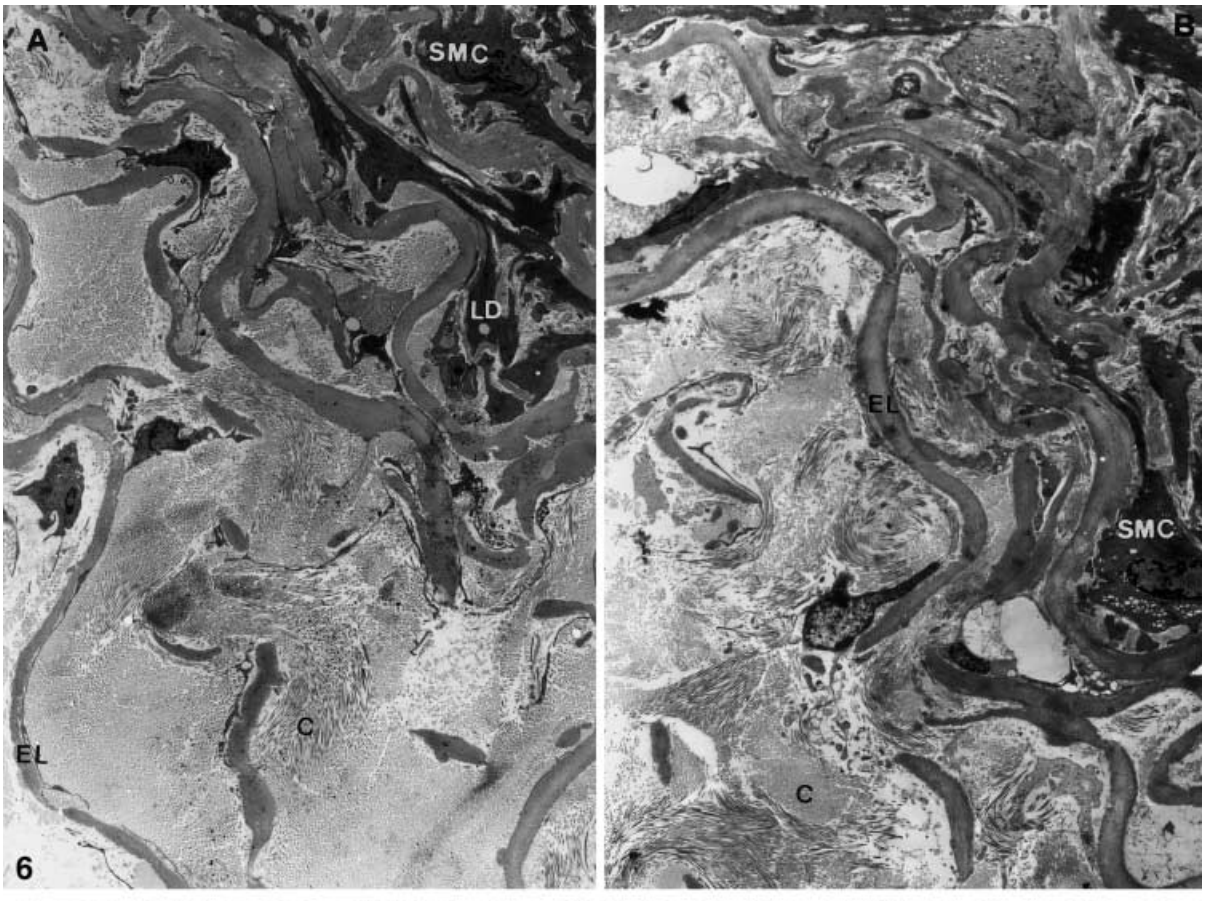


Fig. 4 Orcein-stained sections of aorta from untreated (**A**) or atorvastatin-treated (**B**) rabbits fed a diet containing 0.5% cholesterol+14% coconut oil. **A** In untreated rabbits, media layer presents an important disorganization showing numerous ruptures. The internal elastic layer is duplicate in certain areas. **B** Atorvastatin treatment significantly reduced media disorganization. Original magnification ×64

Fig. 5 Thin-section electron micrographs showing the intima and internal media (**A**) and external layers (**B**) in control rabbits. **B** The three external elastic layers (*EL*) lie together and only a few smooth muscle cells are found among them. *C* collagen fibers, *EC* endothelial cell, *IEL* internal elastic layer, *L* aortic lumen, *SMC* smooth muscle cell. Original magnification ×2400 (**A**) and ×1200 (**B**)


A
SMC
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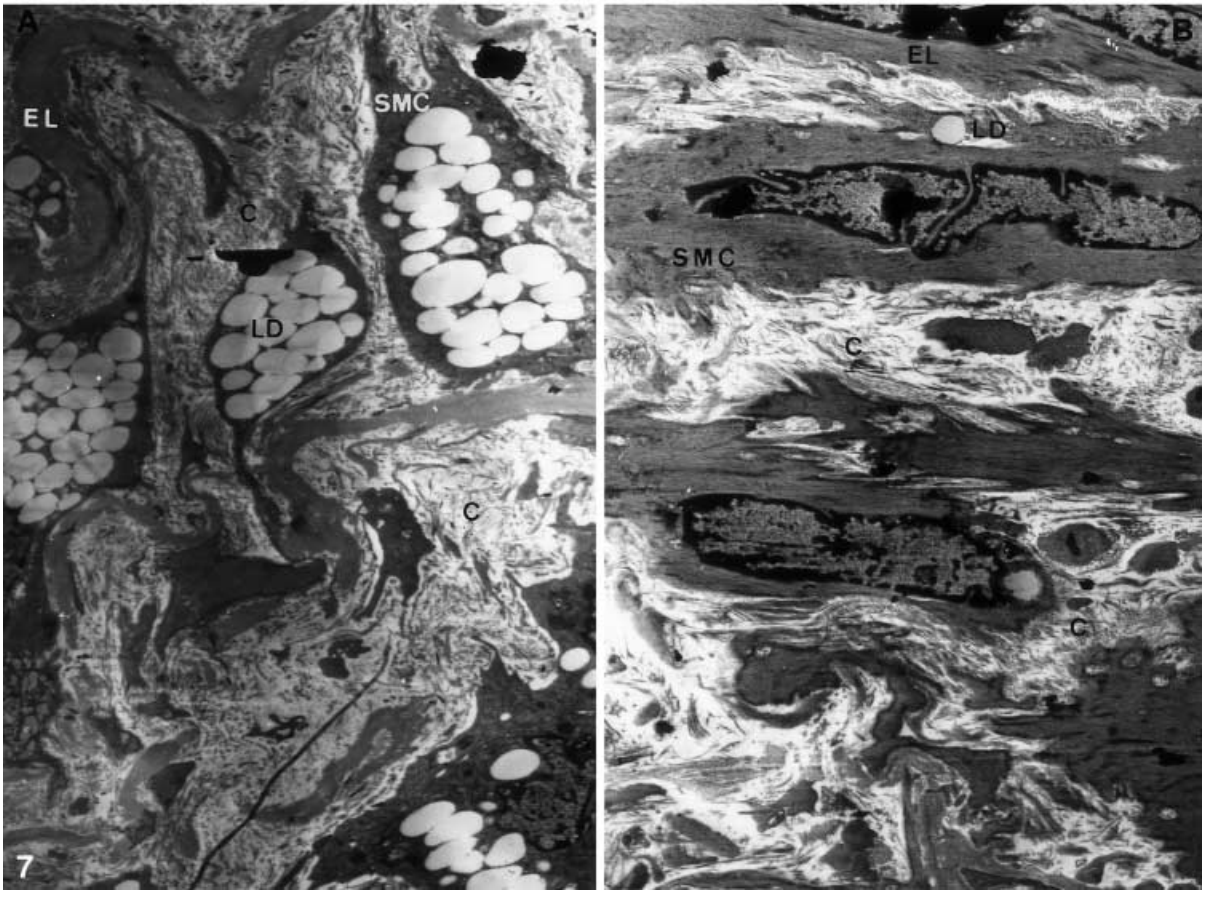
A
EL
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7
B
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SMC
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the lesion areas and the endothelial cells showed important alterations. They were rounded with a polygonal nucleus and presented only a few digitates, which were sometimes broken (Fig. 3A). Likewise, their intercellular junctions showed numerous gaps (Fig. 3A), and they presented a certain degree of edema and endoplasmatic reticulum dilatation and some lipid inclusions (Fig. 3A). In the area of lesions, the internal elastic lamina presented ruptures and was duplicate (Fig. 4A). Atorvastatin treatment prevented the majority of the changes observed in endothelial cells and maintained endothelial integrity. Likewise, atorvastatin not only reduced the lipid inclusions in smooth muscle cells, but also their number contained in the intima and, to a lesser extent, collagen fiber content. In addition, atorvastatin administration reduced the number of foam cells, the extracellular lipid pools and T-cells (Fig. 3B).

No differences were observed in the number of elastic layers [20] in the media between normo- and dyslipidemic rabbits. In contrast to what was observed in control animals were the three external layers lying together and only a few cells being found among them (Fig. 5B). In dyslipidemic rabbits, the layers were separated and had abundant collagen fibers and a few smooth muscle cells present among them (Fig. 6A). In fact, overall media thickness was bigger in aorta from dyslipidemic rabbits than from control ones (152±13 μm versus 116±5 μm, $P<0.05$). Moreover, in dyslipidemic rabbits, the elastic layers presented numerous ruptures (Fig. 4A) and showed larger thickness variability than control ones, not only between animals (range 100–193 μm versus 104–136 μm) but also along the vessel (range: 99–275 μm versus 73–139 μm) (Fig. 2A). No differences were observed in the size of smooth muscle cells between dyslipidemic and control rabbits (3.2±0.15 μm versus 3.4±0.3 μm), although dyslipidemic rabbits showed a gradual outward pattern of phenotype modification. The inner ones (3–4 layers) presented a synthetic phenotype with ovoid shape and abundant rough endoplasmatic reticulum and prominent Golgi apparatus and many lipid inclusions (Fig. 7A). Indeed, numerous foam cells derived from smooth cells were evident in the inner media. The external ones showed a contractile phenotype with only a few lipid inclusions (Fig. 6A). In addition, there was an important increase in the extracellular matrix, especially in the three external elastic layers (Fig. 6A). Atorvastatin treatment did not modify the media thickness (162±6 μm), although it was able to reduce the variability not only between animals (range 146–192 μm) but also along the vessel (range 130–200 μm, Fig. 2B). In addition, this treatment slightly reduced collagen content, especially in the external media layer (Fig. 6B). Likewise, it reduced both lipid inclusions and the presence of foam cells, and it partially prevented changes in the phenotype of smooth muscle cells (Fig. 7B) without modifying their size (3.5±0.1 μm). The ultrastructure of aorta from animals treated with atorvastatin and fed a control diet was comparable to that observed in control animals.

◀ **Fig. 6** Thin-section electron micrographs showing the external media layer from untreated (**A**) or atorvastatin-treated (**B**) rabbits fed a diet containing 0.5% cholesterol+14% coconut oil. In untreated rabbits, the three external elastic layers are separated and with abundant collagen fibers (*C*) and a few smooth muscle cells (*SMC*) present among them containing a few lipid droplets (*LD*). Atorvastatin-treated animals present a similar pattern, although both the distance between the layers and the C content are reduced. *EL* elastic layer; Original magnification ×1200

Fig. 7 Thin-section electron micrographs showing the internal media layer from untreated (**A**) or atorvastatin-treated (**B**) rabbits fed a diet containing 0.5% cholesterol+14% coconut oil. In untreated rabbits, smooth muscle cells (*SMC*) present a synthetic phenotype with ovoid shape and many lipid droplets (*LD*). Atorvastin treatment reduces lipid inclusions and prevents changes in the phenotype of SMC. *C* collagen fibers, *EL* elastic layer. Original magnification ×2400

Discussion

The present study shows that in dyslipidemic rabbits, atorvastatin treatment was not only able to reduce the size of the lesion in the proximal aorta, but also to prevent the majority of the structural and ultrastructural changes observed in endothelial cells. Likewise, atorvastatin administration reduced media disorganisation, but not thickening. In addition, it ameliorated the vasorelaxing response to acetylcholine. All these atorvastatin-induced effects were observed with very high cholesterol and triglyceride plasma levels (30-fold and 3-fold over the controls, respectively). This suggests that this HMG-CoA reductase inhibitors can prevent morphological and functional changes in the vascular wall even in the presence of high plasma lipid levels.

The formation of atherosclerotic lesions is a very complex process, one of the initial steps being an endothelial cell vascular permeability alteration that allows the accumulation and further oxidation of LDL in the artery wall [8, 33]. These permeability changes might be due to high plasma lipids, since it has been shown that exposure of endothelial cells to lipids causes membrane fluidity alterations [1], although an intramural stress increase in the vascular wall has also been suggested [5]. In addition, oxidized LDL can further induce changes in endothelial cell function [39]. Indeed, aortic rings from dyslipidemic rabbits presented altered vascular responses to an endothelium-dependent vasodilator such as acetylcholine, but not to an endothelium-independent agent like sodium nitroprusside, a fact previously reported in both hyercholesterolemia and atherosclerosis [15, 23, 28, 45]. This altered response to endothelium-dependent agents could be a consequence not only of a minor NO availability [22, 24], but also of an increase in vasoconstrictor factors such as endothelin and thromboxane A_2, which can counteract the effect of vasorelaxing factors [11, 17]. The minor response to acetylcholine can also be attributed to intimal thickening, which produces a structural barrier preventing NO from reaching smooth muscle cells.

In agreement with previous reports [2, 10, 13], the present findings show an aortic vessel wall enlargement in dyslipidemic rabbits, the consequence of an intima–media thickening. This effect was associated with a reduction in aortic lumen, since no changes were observed in the vessel area. Similarly, a reduction of aortic lumen has been observed in genetically hyperlipidemic mice and rabbits [10, 18]. By contrast, luminal narrowing is not a common feature in human coronary and renal arteries because intimal thickening is associated with vessel wall outward displacement as a means of preserving the lumen [20, 30]. A possible explanation for this discrepancy could lie in the differences between species. However, the differences could also lie in the studied vessels, since it has been shown that arterial remodelling and the consequent influences on lumen differ among artery types.

Cellular component proliferation, especially foam cells derived from monocytes–macrophages, can account for the intimal thickening observed in dyslipidemic rabbits. These monocytes can enter into the subintima through the numerous gaps or ruptures in their intercellular junctions observed in dyslipidemic rabbits, a common alteration reported in atherosclerosis [6, 33]. In addition, we and others have found a smooth muscle cell accumulation that could also be underlying the intima thickness. These cells can migrate from media to subintimal space through the observed ruptures in the internal elastic lamina in dyslipidemic rabbits, although a proliferation process has also been suggested [31, 33]. Medial smooth muscle cell migration to the intima in response to a wall injury such as atherosclerosis is accompanied by marked morphological and functional changes in the cells [35]. In fact, the intimal smooth muscle cells presented a synthetic phenotype, which can account for the collagen fiber accumulation also observed in the subintima. Likewise, the cells presented lipid inclusions. Indeed, some of the foam cells of the intima are derived from smooth muscle cells that can be present in advanced atherosclerotic lesions [32].

In addition to the blood-derived lipids within the intimal cells, the aorta of dyslipidemic rabbits presented extracellular lipid deposits in the form of liposomes and cholesterol crystals. Subendothelial accumulation of extracellular liposomes formed by plasma lipoprotein fusion has been described as an early feature of atherosclerosis in cholesterol-fed rabbits even before monocyte infiltration happens [21]. However, their specific role in the development of the lesion is not totally established. In agreement with previous studies, we have found cholesterol crystal deposits in the intimal lesions of dyslipidemic rabbits. Since they can be associated with cellular debris, they could be released from dead foam cells.

Atherosclerosis is considered to be a chronic inflammatory state [8]. Indeed, there has been shown to be a T-cell accumulation in the intimal lesions that seems temporally correlated with macrophage proliferation, suggesting that it can participate in this process [19]. This accumulation seems to be greater in the first weeks of lesion development. The accumulation then decreases with time, therefore suggesting that the scarce lymphocytes found in the dyslipidemic rabbit lesions could be a consequence of timing.

The present data show that in dyslipidemic rabbits, the endothelium was not always overlying the lesions as a consequence of the huge subintimal area increase. In addition, the endothelial cells presented not only functional but also morphological changes. In fact, the cells of the proximal aorta of these animals showed a rounded shape as well as intercellular junction alterations. These changes could be due to local mechanical forces such as intramural stress and shear stress that can affect the atherosclerosis-prone vessels such the proximal aorta [5, 6]. In addition, endothelial cells presented edema and lipid inclusions. These alterations could be the consequence of the previously mentioned changes in their barrier function due to high plasma lipids [1]. Moreover, endothelial cells showed an endoplasmatic reticulum dilatation supporting an increase in synthetic activity.

In dyslipidemic rabbits, the media cross-sectional area increase seems mainly to be a consequence of collagen fiber accumulation of extracellular matrix and not due to smooth muscle cell proliferation or hypertrophy since no changes in smooth muscle cell number or size were observed. Collagen fibers can be synthesized by smooth muscle cells in the synthetic phenotype observed in the media. Indeed, smooth muscle presented a gradual outward pattern phenotype modification, since the external ones showed a contractile phenotype and the inner ones a synthetic one. This suggests a graded exposition of the cells to phenotype modification-induced factors. It is important to note that although the structure of the media layer appears to be affected by the dyslipidemia, smooth muscle cells presented a response to sodium nitroprusside similar to that of control animals.

As expected, the present data show that the administration of atorvastatin, an HMG-CoA reductase inhibitor, reduced cholesterol levels in dyslipidemic rabbits. This effect seems to be the consequence of both a decrease in cholesterol synthesis and an increase in the LDL receptor expression [9]. In addition, atorvastatin reduced triglyceride levels, a feature previously reported [42]. This effect might be due to its ability to modulate the expression of proteins involved in triglyceride metabolism [27]. In addition, atorvastatin was also able to reduce the aortic wall lipid content not only by decreasing the extracellular but also the intracellular deposits in both intima and media layers. In agreement with this observation, it has been reported that fluvastatin therapy reduced platelet cholesterol content in hypercholesterolemic patients [29]. This effect could be due to a plasma lipid reduction and consequently a minor artery wall retention. However, taking into consideration that even after the atorvastatin-induced reduction (31%) lipid plasma levels were high enough to expect changes in vascular permeability suggests that additional mechanisms could be underlying this effect.

Atorvastatin treatment also reduced lesion size, a fact previously reported using HMG-CoA inhibitors [4, 12,

14, 18, 38]. While this effect seems to be the consequence of a reduction in all its components, it is mainly due to a decrease in cell content (including T-cells) and lipid deposits. Statins inhibit the LDL oxidation [4], monocyte infiltration [14], macrophage growth induced ox-LDL [34], cholesterol accumulation in macrophages [7] and smooth muscle cell migration and proliferation [7, 16]. Therefore, all these effects can account for this beneficial effect of atorvastatin. Likewise, atorvastatin treatment was also able to prevent the endothelial cell ultrastructure changes produced by dyslipidemia and maintain endothelium integrity, suggesting a protective role of atorvastatin even in the presence of high lipid plasma levels. The present study also shows that, in dyslipidemic rabbits, atorvastatin treatment prevents endothelial dysfunction, as demonstrated by a response to acetylcholine similar to that of the control group rings. These results are in agreement with previous studies that have shown that lipid-lowering drugs can improve endothelium-dependent relaxation in both humans and animals with dyslipidemia [3, 12, 40, 44]. Moreover, this statin was also able to enhance acetylcholine relaxation in normocholesterolemic rabbits without modifying lipid levels. This suggests that the atorvastatin action on endothelial function is, in part, independent of its hypolipidemic action. Several mechanisms can account for this beneficial effect on endothelial function, including an increase in NO, since statins can upregulate endothelial NO synthase expression and also prevent its downregulation induced by ox-LDL [22, 24]. In fact, it has been shown that lovastatin selectively mantains NO-mediated acetylcholine relaxation in hypercholesterolemic rabbits [12]. In addition, a decrease in vasoconstrictor agent availabilities could be involved, as they can reduce vasoconstrictor factor availabilities [22]. Moreover, this endothelial function amelioration could have also accounted for the observed reduction in intimal thickening.

The present data also show that atorvastatin treatment reduced collagen fiber content in both intima and media layers, although to a lesser extent than it did cellular components, probably as a consequence of the reduction in the number of smooth muscle cells in synthetic phenotype. Although, atorvastatin reduced collagen content, this reduction was not large enough to reduce medial thickness. However, this layer became more homogenous through the elimination of areas of extreme thickness. Since the unstable plaque is characterized by, among other factors, a large lipid-rich core with macrophage and T-cell increases, collagen degradation and smooth muscle cell reduction in critical locations [25, 27], all these data support the concept that atorvastatin can protect against plaque disruption and account for the reduction in coronary events associated with statin treatment [37, 44].

In summary, all these data show that atorvastatin treatment was able to prevent not only functional but also structural and ultrastructural alterations in the vascular wall associated with dyslipidemia. All these effects were observed in animals with high cholesterol and triglyceride plasma levels, suggesting that atorvastatin can exert a protective role on vascular wall. This supports the notion that statins can exert additional vascular mechanisms beyond their lipid-lowering effect, which can explain the early cardiovascular event reduction observed in clinical trials.

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