Original Article

Regression of Atherosclerosis by Amlodipine *via* Anti-Inflammatory and Anti-Oxidative Stress Actions

Toyofumi YOSHII[1)], Masaru IWAI[2)], Zhen LI[2)], Rui CHEN[2)], Ayumi IDE[2)], Shiori FUKUNAGA[2)], Akira OSHITA[1)], Masaki MOGI[2)], Jitsuo HIGAKI[1)], and Masatsugu HORIUCHI[2)]

We examined whether amlodipine, an L-type calcium channel blocker (CCB), has an inhibitory effect on oxidative stress and inflammatory response, and thereby atherosclerosis, in apolipoprotein E–deficient (ApoEKO) mice. Adult male ApoEKO mice (6 weeks of age) were fed a high-cholesterol diet (HCD) for 8 or 10 weeks with or without oral administration of amlodipine (3 mg/kg/day) for 10 weeks or for only the last 2 weeks of the HCD. After HCD feeding, atherosclerotic lesion formation, *in situ* superoxide production and nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase activity were evaluated in the proximal aorta. The expressions of NADPH oxidase subunits ($p47^{phox}$ and rac-1), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) were determined with immunohistochemistry and quantitative real-time reverse-transcription polymerase chain reaction. After 8 to 10 weeks of HCD administration to ApoEKO mice, marked atherosclerotic lesion formation was observed in the proximal aorta. In the atherosclerotic lesion, superoxide production, the expression of NADPH oxidase subunits, and NADPH oxidase activity were enhanced, and the expressions of MCP-1, ICAM-1, and VCAM-1 were increased. These changes were suppressed in mice that were treated with amlodipine for 10 weeks concomitant with HCD administration, with no significant change in blood pressure and plasma cholesterol level. We also observed that treatment with amlodipine for only the last 2 weeks regressed the atherosclerotic lesions with a decrease in oxidative stress and vascular inflammation. Inhibition of the atherosclerotic lesion area and lipid area in the proximal aorta by amlodipine was correlated with its inhibitory actions on oxidative stress, inflammation and the production of adhesive molecules. These results suggest that amlodipine not only inhibits atherosclerotic lesion formation, but also regresses atherosclerosis, and that these effects are at least partly due to inhibition of oxidative stress and inflammatory response. (*Hypertens Res* 2006; 29: 457–466)

***Key Words*: atherosclerosis, calcium channel blocker, inflammation, oxidative stress**

Introduction

L-type calcium channel blockers (CCBs) are widely used in the treatment of both hypertension and coronary heart disease (*1*, *2*). In the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT), the CCB amlodipine reduced the rate of cardiac events and inhibited the progression of carotid artery atherosclerosis (*3*). Such vasoprotective effects of CCBs are mediated, at least in part, by an

From the [1)]Second Department of Internal Medicine and [2)]Department of Molecular and Cellular Biology, Division of Medical Biochemistry and Cardiovascular Biology, Ehime University School of Medicine, Tohon, Japan.

Address for Reprints: Masatsugu Horiuchi, M.D., Ph.D., Department of Molecular and Cellular Biology, Division of Medical Biochemistry and Cardiovascular Biology, Ehime University School of Medicine, Shitsukawa, Tohon, 791–0295 Japan. E-mail: horiuchi@m.ehime-u.ac.jp

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improvement of endothelial dysfunction, including the restoration of nitric oxide production. Zhang and Hintze reported that amlodipine increased nitric oxide generation in canine coronary vessels (*4*). However, the detailed mechanism of the inhibitory effects of CCBs on atherosclerosis formation is not entirely clear. Moreover, the question of whether CCBs might actually retard atherosclerosis has not been adequately investigated.

The production of reactive oxygen species (ROS; *e.g.*, superoxides, peroxynitrite, *etc*.) together with inflammatory factors such as chemokines, cytokines, and adhesion molecules has been shown to be increased in atherosclerotic lesions (*5*, *6*). Nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase consists of plasma membrane subunits and cytosolic subunits and plays an important role in superoxide production. Rac-1 is a small guanosine triphosphate–binding protein of the Ras superfamily that is necessary, along with other components (*e.g.*, $p47^{phox}$, $p22^{phox}$, nox-1, nox-4, *etc*.) for activation of NADPH oxidase (*7*, *8*). Moreover, rac-1 has been shown to mediate the shear-induced tyrosine phosphorylation of mitogen-activated protein kinase *via* regulation of the flow-dependent redox changes in endothelial cells under pathological conditions, such as endothelial dysfunction associated with atherosclerosis (*9*). Some CCBs decrease inflammation (*10*) and oxidative stress (*11*) in atherosclerosis. However, the anti-atherosclerotic action of amlodipine has not yet been well examined. Apolipoprotein E–deficient (ApoEKO) mice are commonly used to study the pathogenesis of human atherosclerosis (*12*). In this study, we used these mice to examine the effects of amlodipine on the progression and possible regression of atherosclerosis, with particular focus on the anti-inflammatory and anti-oxidative stress actions.

Methods

Animals and Treatment

Adult male ApoEKO mice (6 weeks of age; Jackson Laboratory, Bar Harbor, USA) received a standard normal diet (ND) (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) or high-cholesterol diet (HCD; 1.25% cholesterol, 10% coconut oil in MF) for 8 or 10 weeks and water ad libitum (*13*). Amlodipine (UK-48340; donated by Pfizer Inc., New York, USA) was administered daily by gavage at a dose of 3 mg/kg/day for 10 weeks. In some mice, amlodipine was administered for only the last 2 weeks of HCD administration. There were thus 5 groups: ND; HCD for 8 weeks; HCD for 10 weeks; HCD for 10 weeks + amlodipine for 2 weeks; and HCD for 10 weeks + amlodipine for 10 weeks. The plasma cholesterol concentration and systolic blood pressure (SBP) were measured as described previously (*13*). The Animal Studies Committee of Ehime University approved the experimental protocol of this study.

Table 1. Systolic Blood Pressure and Plasma Cholesterol Level in ApoEKO Mice

	SBP (mmHg)	Cholesterol (mg/dl)
ND	99.5±0.7	619.3±19.6
HCD for 8 weeks	100.4±1.5	1,153.3±47.5*
HCD for 10 weeks	100.5±1.4	1,147.1±42.0*
HCD for 10 weeks + Aml for 2 weeks	99.8±1.1	1,194.6±65.9*
HCD for 10 weeks + Aml for 10 weeks	98.7±1.0	1,216.0±49.8*

ApoEKO mice were treated with HCD and amlodipine was given as described in Methods. ApoEKO mice, apolipoprotein E–deficient mice; SBP, systolic blood pressure; ND, normal standard diet; HCD, high-cholesterol diet; Aml, amlodipine at 3 mg/kg/day. Values are the mean±SEM. $n=7$ for each group. *$p<0.05$ *vs*. the ND group.

Atherosclerotic Lesion Size

The area of atherosclerotic lesions in the proximal aorta and the lipid area in the aortic wall and the atherosclerotic lesions were determined by taking intermittent cross sections throughout a 2–3 mm length piece of the ascending aorta, followed by oil-red O staining and counterstaining with hematoxylin (*14*). Quantitative analysis was performed with Densitograph imaging software (ATTO Corporation, Tokyo, Japan). The mean value of 5 sections was taken as the value for each animal.

In Situ Detection of Superoxide Production

Superoxide production was detected in the proximal aorta using freshly frozen sections stained with dihydroethidium (10 μmol/l), and the intensity of fluorescence was measured as previously described (*15*).

NADPH Oxidase Activity

A tissue protein sample was prepared from the aorta after homogenization in 500 μl ice-cold Tris-sucrose buffer (*16*). NADPH oxidase activity was quantified by the cytochrome c method from the absorbance with or without superoxide dismutase (SOD), as previously described (*16*).

Real-Time Reverse-Transcription Polymerase Chain Reaction

Quantitative real-time reverse-transcription polymerase chain reaction (PCR) was performed using Premix Ex Taq™ (Takara Bio Inc., Shiga, Japan). The PCR primers for monocyte chemoattractant protein-1 (MCP-1) were 5′-TTAACG CCCCACTCACCTGCTG-3′ (forward) and 5′-GCT

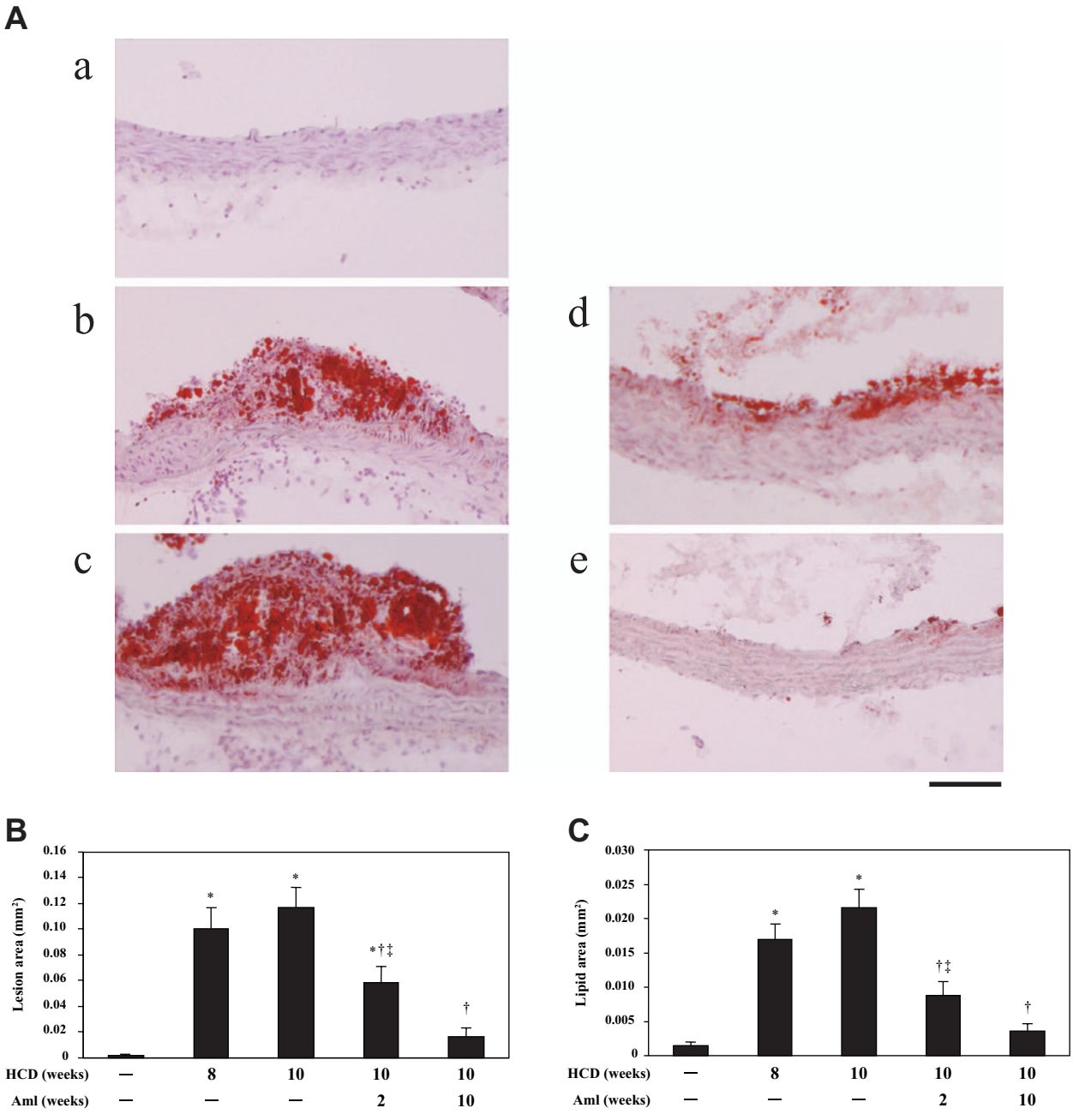

Fig. 1. *Atherosclerotic lesions of the proximal aorta from apolipoprotein E–deficient (ApoEKO) mice fed a high-cholesterol diet (HCD). A: Representative oil red O–stained cross-section of the proximal aorta of ApoEKO mice showing lipid deposition. a: Normal standard diet (ND); b: HCD for 8 weeks; c: HCD for 10 weeks; d: HCD for 10 weeks + 3 mg/kg/day amlodipine for the last 2 weeks; e: HCD for 10 weeks + 3 mg/kg/day amlodipine for 10 weeks. Magnification: ×200. The scale bar represents 100 μm. B: Morphometry of atherosclerotic area in cross-sections of the proximal aorta. C: Morphometry of lipid accumulation in cross-sections of the proximal aorta. The mean value of 5 sections was taken as the value for each animal. Aml, amlodipine (3 mg/kg/day). Values are the mean±SEM (*n = 7 or 8 per group). *$p < 0.05$ vs. *ND*, †$p < 0.05$ vs. *HCD for 10 weeks*, ‡$p < 0.05$ vs. *HCD for 8 weeks.*

TCTTTGGGACACCTGCTGC-3′ (reverse); those for rac-1 were 5′-CCAGTGAATCTGGGCCTATG-3′ (forward) and 5′-ACAGTGGTGTCGCACTTCAG-3′ (reverse); those for $p47^{phox}$ were 5′-GTCCCTGCATCCTATCTGGA-3′ (forward) and 5′-GGGACATCTCGTCCTCTTCA-3′ (reverse); those for intercellular adhesion molecule-1 (ICAM-1) were

5′-GGCACCCAGCAGAAGTTGTT-3′ (forward) and 5′-CCTCAGTCACCTCTACCAAG-3′ (reverse); those for vascular cell adhesion molecule-1 (VCAM-1) were 5′-TCT CTCAGGAAATGCCACCC-3′ (forward) and 5′-CACAGC CAATAGCAGCACAC-3′ (reverse); and those for glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were 5′-TGCGACTTCAACAGCAACTC-3′ (forward) and 5′-ATG TAGGCCATGAGGTCCAC -3′ (reverse).

Immunofluorescent Staining of p47^{phox} in Atherosclerotic Lesions

Immunofluorescence was assessed using freshly frozen sections that were incubated with anti-p47^{phox} antibody, washed and incubated with biotin-labeled secondary antibodies, and then incubated with Cy3-labeled streptavidin. Serial sections treated with secondary antibodies alone did not show specific staining. Samples were examined with a Zeiss Axioskop microscope equipped with a computer-based imaging system (*13*).

Data Analysis

All values are expressed as the mean±SEM. The data were analyzed using one-way ANOVA. If a statistically significant difference was found, Newman-Keuls' test was performed for post-hoc analysis to detect the differences among groups. Pearson's correlation coefficient was used to assess the correlation between the atherosclerotic lesion area or lipid area and each of the markers for oxidative stress, inflammation and adhesive molecules. Values of $p<0.05$ were considered statistically significant.

Results

Inhibitory Effect of Amlodipine on Atherosclerotic Lesion Formation in ApoEKO Mice Fed a High-Cholesterol Diet

In the preliminary experiments, we administered amlodipine at a dose of 0.5 or 3.0 mg/kg/day for 10 weeks. Neither dose affected the SBP in ApoEKO mice. However, a dose of 3.0 mg/kg/day but not 0.5 mg/kg/day inhibited atherosclerotic lesion formation. Therefore, in the following experiments, we used amlodipine at 3.0 mg/kg/day. Plasma cholesterol levels in ApoEKO mice were markedly increased after administration of the high-cholesterol diet ($p<0.05$ *vs*. the ND group; Table 1). After 8 or 10 weeks of the HCD diet, atherosclerotic lesion formation with lipid accumulation was observed in the proximal aortas of ApoEKO mice (Fig. 1). Administration of amlodipine (3 mg/kg/day) concomitant with HCD for 10 weeks markedly inhibited atherosclerotic lesion formation in ApoEKO mice (Fig. 1), but did not affect the SBP or plasma cholesterol level (Table 1). Interestingly, even when amlodipine was administered for only the last 2 weeks of the 10-week HCD, the atherosclerotic lesions that had already formed were significantly reduced ($p<0.05$ *vs*. HCD alone for 8 or 10 weeks, respectively; Fig. 1).

Effect of Amlodipine on Oxidative Stress and Inflammatory Response

Figure 2A shows the results of the *in situ* detection of superoxide production by dihydroethidium in the proximal aorta. The measured fluorescence intensities are shown in Fig. 2B. Superoxide production was increased by HCD treatment for 8 or 10 weeks (Fig. 2A, B). We also observed an increase in NADPH oxidase activity in atherosclerotic lesions by HCD feeding (Fig. 2C). Expression of p47^{phox}, a subunit of NADPH oxidase, detected by immunofluorescence was also increased in atherosclerotic lesions and the aortic wall in the proximal aorta after HCD treatment (Fig. 3). The expressions of the mRNAs of p47^{phox} and rac-1 were also increased in the atherosclerotic aorta (Fig. 4A, B). Treatment with amlodipine for 10 weeks inhibited the increase in superoxide production, NADPH oxidase activity, and expression of p47^{phox} and rac-1 in atherosclerotic lesions. Moreover, treatment with amlodipine for only the last 2 weeks of HCD feeding also decreased superoxide production, NADPH oxidase activity, and expression of p47^{phox} and rac-1 in atherosclerotic lesions.

In addition, the expressions of MCP-1, ICAM-1, and VCAM-1 were increased by HCD treatment (Fig. 4C–E). Administration of amlodipine for 10 weeks inhibited these changes. Amlodipine also decreased the increased expression of MCP-1, ICAM-1, and VCAM-1 when it was administered for only the last 2 weeks of the 10-week HCD feeding (Fig. 4C–E). Moreover, as shown in Table 2, three of the groups, *i.e.*, the ND, 10-week HCD, and 10-week HCD + 10-week amlodipine groups, showed a significant correlation between both the atherosclerotic lesion area and the lipid area in the proximal aorta and each of superoxide production and p47^{phox}, rac-1, MCP-1, ICAM-1 and VCAM-1 expression.

Discussion

We have demonstrated that simultaneous administration of amlodipine with HCD feeding for 10 weeks inhibited atherosclerotic lesion formation in ApoEKO mice, without affecting the SBP or plasma cholesterol level. Moreover, even when amlodipine was administered for only the last 2 weeks of the 10-week HCD, the size of the atherosclerotic lesions was significantly reduced. These inhibitory actions of amlodipine were accompanied by inhibitions of oxidative stress and the inflammatory response. It appears that this type of inhibitory action on atherosclerosis is not specific to amlodipine, since similar effects have been observed in experimental studies with the CCBs azelnidipine (*11*) and lacinidipine (*17*, *18*), as well as in a clinical study with these agents (*19*).

Previous papers reported that atherosclerotic lesions were formed in ApoEKO mice given a regular (normal) diet for a

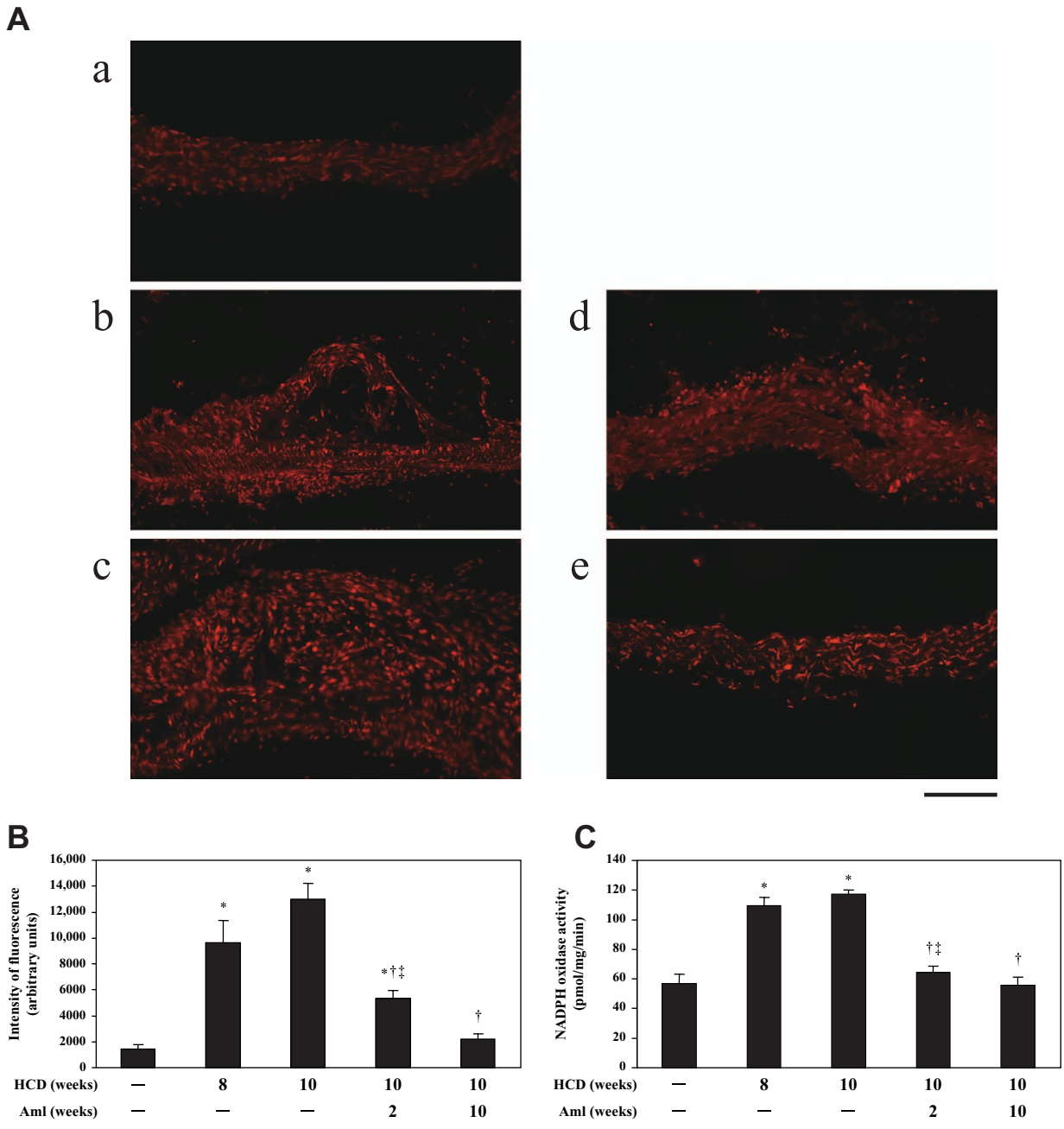

Fig. 2. In situ *detection of superoxide production in the proximal aorta of apolipoprotein E–deficient (ApoEKO) mice treated with high-cholesterol diet (HCD). Animals were treated and aortic samples were taken as described in Fig. 1. A: Representative detection of superoxide production with dihydroethidium staining in cross-sections of the proximal aorta of ApoEKO mice. a: Normal standard diet (ND); b: HCD for 8 weeks; c: HCD for 10 weeks; d: HCD for 10 weeks + 3 mg/kg/day amlodipine for the last 2 weeks; e: HCD for 10 weeks + 3 mg/kg/day amlodipine for 10 weeks. Magnification: ×200. The scale bar represents 100 μm. B: Measurement of fluorescence intensity for superoxide production. The mean value of 3 sections was taken as the value for each animal. Values are the mean±SEM for measurement of intensity (*n = 6 per group). C: NADPH oxidase activity in the atherosclerotic aorta of ApoEKO mice treated with HCD. Aml, amlodipine (3 mg/kg/day); NADPH, nicotinamide-adenine dinucleotide phosphate. Values are the mean±SEM (n = 6 or 7 per group). *$p < 0.05$ vs. *ND*, †$p < 0.05$ vs. *HCD for 10 weeks*, ‡$p < 0.05$ vs. *HCD for 8 weeks*.

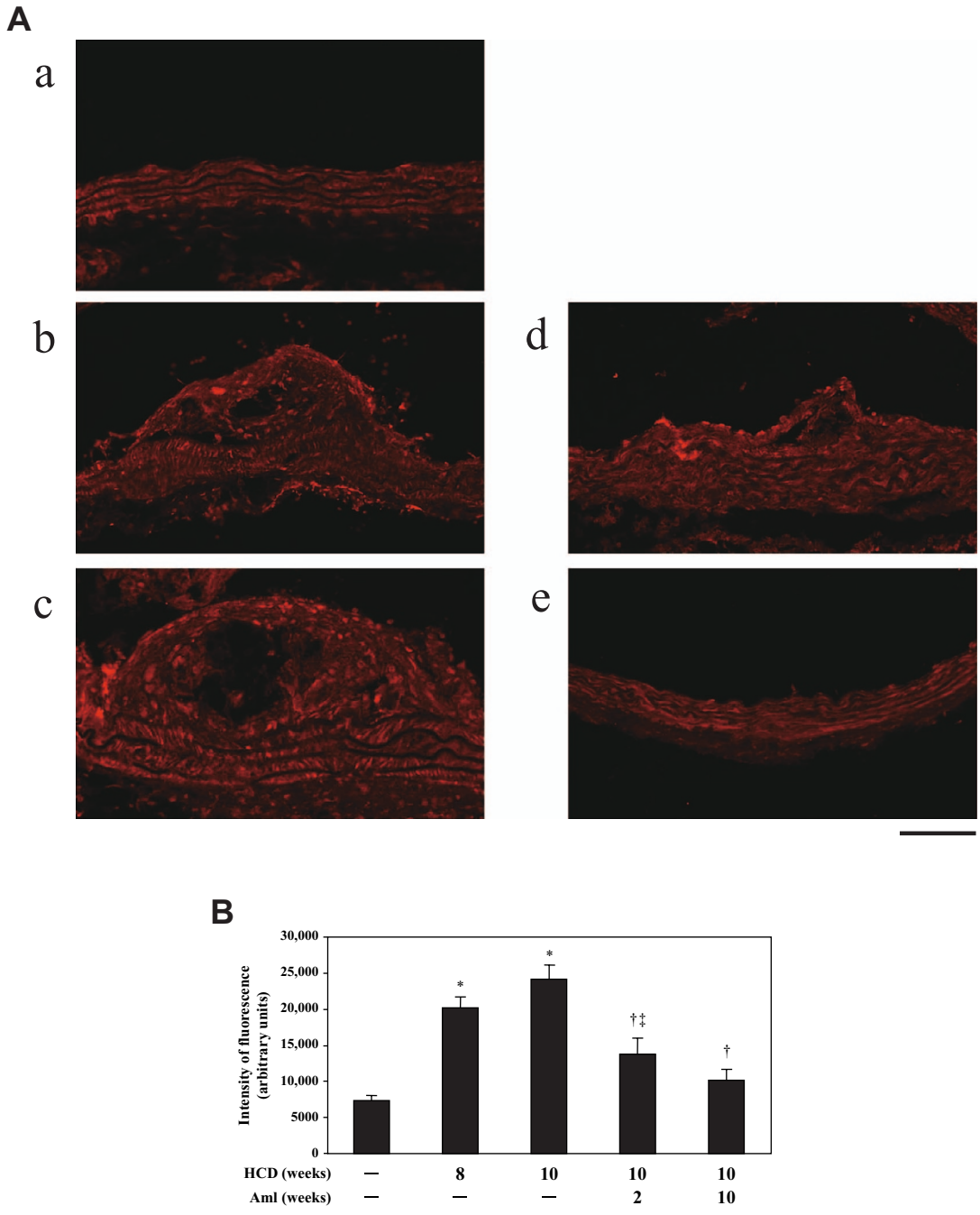

Fig. 3. *Immunohistochemical staining of p47^{phox} in the proximal aorta of apolipoprotein E–deficient (ApoEKO) mice treated with high-cholesterol diet (HCD). Animals were treated and aortic samples were taken as described in Fig. 1. A: Representative immunostaining of p47^{phox} in cross-sections of the proximal aorta of ApoEKO mice. a: Normal standard diet (ND); b: HCD for 8 weeks; c: HCD for 10 weeks; d: HCD for 10 weeks + 3 mg/kg/day amlodipine for the last 2 weeks; e: HCD for 10 weeks + 3 mg/kg/day amlodipine for 10 weeks. Magnification: ×200. The scale bar represents 100 μm. B: Measurement of fluorescence intensity for p47^{phox}. The mean value of 3 sections was taken as the value for each animal. Aml, amlodipine (3 mg/kg/day). Values are the mean±SEM for measurement of intensity (*n = 6 per group). *$p < 0.05$ vs. *ND*, †$p < 0.05$ vs. *HCD for 10 weeks*, ‡$p < 0.05$ vs. *HCD for 8 weeks.*

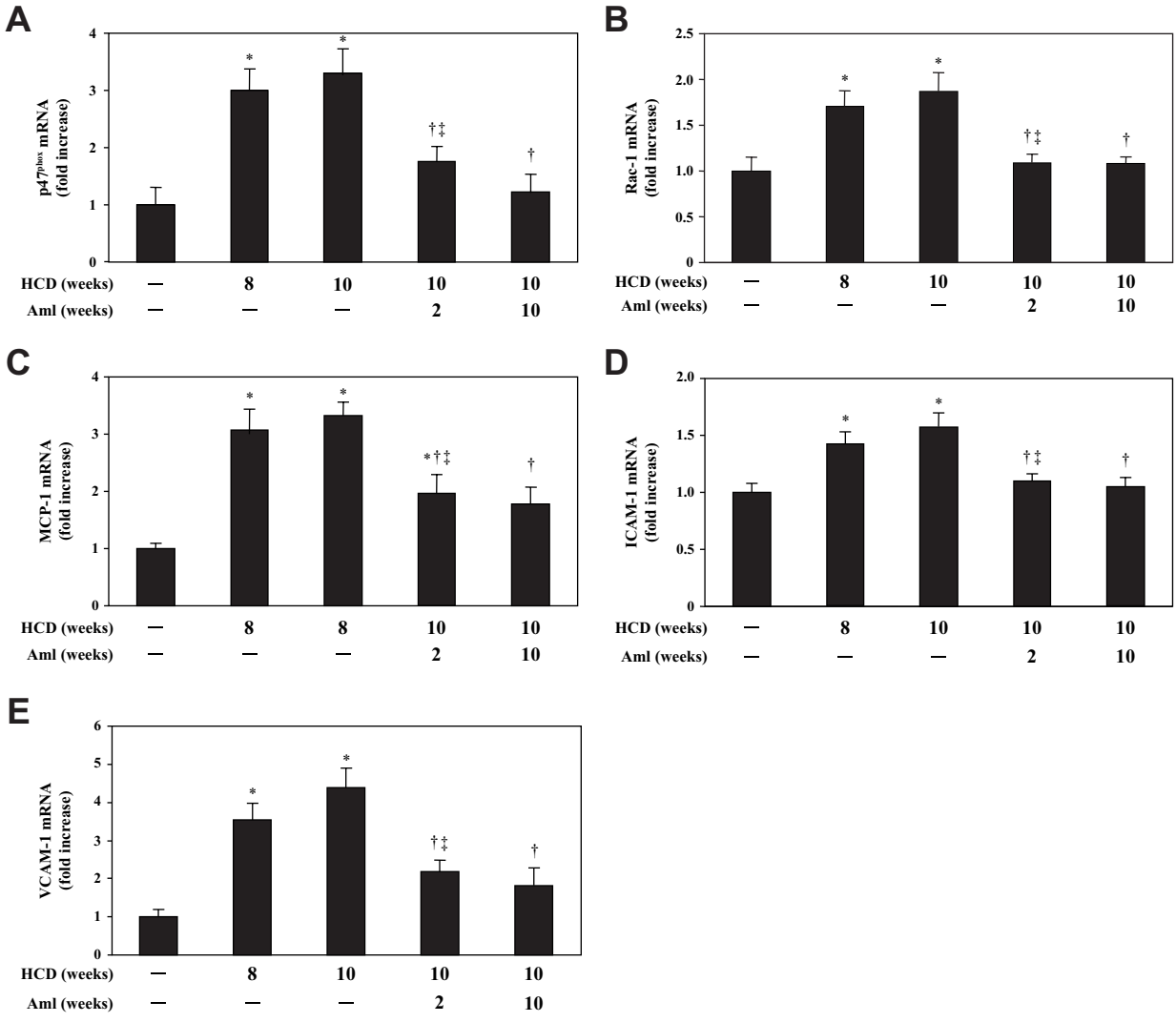

Fig. 4. *Expression of p47^{phox}, rac-1, MCP-1, ICAM-1 and VCAM-1 in the atherosclerotic aorta of apolipoprotein E–deficient (ApoEKO) mice treated with high-cholesterol diet (HCD). Aortic samples were taken from ApoEKO mice as described in the Methods section. Levels of mRNA for p47^{phox} (A), rac-1 (B), MCP-1 (C), ICAM-1 (D) and VCAM-1 (E) were assayed by quantitative real-time reverse-transcription polymerase chain reaction as described in the Methods section. Values are the mean±SEM (*n*=6 to 8 per group).* *p<0.05 vs. *ND,* †p<0.05 vs. *HCD for 10 weeks,* ‡p<0.05 vs. *HCD for 8 weeks. ND, normal standard diet; Aml, amlodipine (3 mg/kg/day); MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.*

longer period, like 20 weeks (*20*) or 30 weeks (*21*). However, in our study, the atherosclerotic lesions were not significantly formed in ApoEKO mice given a regular diet for 10 weeks, and it was not examined whether similar inhibitory changes by amlodipine are observed in ApoEKO mice treated with regular diet for a longer feeding period. Other research groups also used high-cholesterol diet or Western-style diet to ApoEKO mice to make atherosclerotic lesions, and they observed no apparent atherosclerosis with regular diet in ApoEKO mice (*22*). The reason for such a discrepancy of the atherosclerotic lesion formation in ApoEKO mice by regular diet is not yet clear, however, the discrepancy may depend on 1) the difference of nutritional component of regular (normal) diet from different commercial sources, and/or 2) the different feeding period of regular diet in ApoEKO mice.

There are controversial findings in regard to the effect of amlodipine on atherosclerosis. Candido *et al*. (*20*) reported that amlodipine did not significantly reduce atherosclerosis even at a hypotensive dose (6 mg/kg/day for 20 weeks) in diabetic ApoEKO mice. They reported that in the ApoEKO mice treated with streptozotocin, the vascular renin-angiotensin system plays a critical role in mediating acceleration of ath-

Table 2. Correlation between Atherosclerotic Lesion Area, Lipid Area and the Markers for Oxidative Stress and Inflammation and Adhesive Molecules

	Correlation coefficient	
	Lesion area	Lipid area
Superoxide production	0.972‡	0.913‡
p47^{phox} mRNA expression	0.715†	0.623*
Rac-1 mRNA expression	0.877‡	0.871‡
MCP-1 mRNA expression	0.818‡	0.732†
ICAM-1 mRNA expression	0.886‡	0.874‡
VCAM-1 mRNA expression	0.867‡	0.868‡

MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1. *$p<0.01$,†$p< 0.001$,‡$p<0.0001$.

erosclerosis, and this may explain why irbesartan was superior to amlodipine in reducing the diabetes-associated acceleration of atherosclerosis. Takai *et al*. (*23*) reported that amlodipine tended to decrease the atherosclerotic area in monkeys fed a high-cholesterol diet, although this effect was not statistically significant. In contrast, others have reported that amlodipine inhibited atherosclerosis in non-diabetic animal models of atherosclerosis (*24*, *25*). These apparently conflicting results may be partly related to the different animal models (diabetic or non-diabetic ApoEKO mice) or different species (mice or monkeys) used.

The results of a clinical trial, the CAMELOT study, showed that amlodipine tended to inhibit the progression of plaque formation in the coronary arteries (*26*). This result, like some of those described above, suggests that amlodipine has an inhibitory effect on atherosclerosis. Moreover, our results suggest that amlodipine not only inhibits atherosclerosis, but also induces regression of atherosclerosis. These inhibitory actions of amlodipine seem to be associated with inhibition of inflammatory responses and oxidative stress. Amlodipine has also been shown to be effective in improving left ventricular hypertrophy by controlling blood pressure (*27*). Inflammation plays an important role in atherosclerosis formation (*5*, *28*). In our study, the expressions of MCP-1, ICAM-1, and VCAM-1 were increased by HCD treatment (Fig. 4C–E). Oxidative stress is also an important factor in inflammatory responses (*7*, *29*). Previous reports indicated that markers of oxidative stress, such as NADPH oxidase activity, were increased in atherosclerotic lesions (*8*, *21*). NADPH oxidase consists of subunits of membrane components (Nox-1, Nox-4, and p22^{phox}) and cytosolic components (p47^{phox} and rac-1, *etc*.). In our study, superoxide production, NADPH oxidase activity, and the expression of the cytosolic components p47^{phox} and rac-1 were markedly increased in atherosclerotic lesions (Figs. 2, 3, and 4A, B). Li *et al*. reported that both calcium influx through receptor-regulated channels and mobilization of intracellular calcium were crucial events for oxidation of lipids in monocytes, a superoxide anion–dependent event (*30*). In our study, amlodipine inhibited the expression of MCP-1, ICAM-1 and VCAM-1, and oxidative stress, such as that related to superoxide production and NADPH oxidase activation, when administered for only the last 2 weeks as well as for all 10 weeks of HCD feeding (Figs. 2, 4C–E).

There was no significant difference in NADPH oxidase activity and the expressions of p47^{phox} fluorescence, rac-1, MCP-1, ICAM-1, or VCAM-1 between the 2-week amlodipine treatment group and 10-week amlodipine treatment group (Figs. 3B, 4B–E). These results seem to be discrepant from those that the inhibition of atherosclerotic lesions, lipid deposition, and superoxide production by amlodipine were stronger in the 10-week amlodipine group than in the 2-week amlodipine group (Figs. 1B, C and 2B). In a previous report, amlodipine was found to preserve SOD activity and reduce the oxidizability of low density lipoproteins (*31*). It has also been reported that amlodipine reduces oxidative stress by restoring copper/zinc-containing SOD activity in the heart in hypertensive rats (*32*). Moreover, amlodipine has been shown to increase nitric oxide (NO) production *via* stimulation of the production of endothelial nitric oxide synthase (NOS) (*33*, *34*) and to inhibit the infiltration of monocytes/macrophages into atherosclerotic lesions (*24*) and the expression of collagens type I, III, and IV (*35*). In the present study, these anti-atherosclerotic actions of amlodipine may have played a role in the changes in atherosclerotic lesions, lipid deposition and superoxide production in the groups administered amlodipine for 2 weeks or 10 weeks.

The mechanism by which amlodipine regresses atherosclerotic lesions is not yet clear. In the present study, administration of amlodipine for the last 2 weeks of the 10-week HCD period reduced the expression of ICAM-1 and VCAM-1, suggesting that amlodipine inhibited the infiltration of monocytes/macrophages into the atherosclerotic lesions. It has been reported that amlodipine decreased the DNA synthesis induced by basic fibroblast growth factor through inhibition of extracellular signal regulated kinase (ERK) activity in human vascular smooth muscle cells (VSMCs) (*36*). It has recently been reported that macrophage apoptosis is associated with diminished lesion cellularity and decreased lesion progression (*37*), and that amlodipine regressed aortic wall hypertrophy in spontaneous hypertensive rats through enhanced apoptosis (*38*), suggesting that apoptosis is involved in the regulation of atherosclerosis. These results suggest that both the inhibition of VSMC proliferation and the enhancement of apoptosis might be involved in the regression of atherosclerotic lesions by amlodipine.

Moreover, a significant correlation was observed between both the atherosclerotic lesion area and the lipid area in the proximal aorta and each of superoxide production and p47^{phox}, rac-1, MCP-1, ICAM-1 and VCAM-1 expression in three experimental groups, *i.e.*, the ND, 10-week HCD, and 10-week HCD + 10-week amlodipine (Table 2). These results also suggest that the inhibitory actions of amlodipine on oxidative stress, inflammation and the production of adhesive

molecules were involved in the inhibition of atherosclerotic lesion area and lipid area induced by amlodipine. To clarify the importance of blockade of NADPH oxidase activity in the inhibitory action of amlodipine, future experiments using an inhibitor of NADPH oxidase might be very useful.

In summary, a CCB, amlodipine, not only inhibited the progression of atherosclerotic lesion formation, but also induced regression of atherosclerotic lesions at least in part due to inhibition of the inflammatory response and oxidative stress. The results of the present study may suggest a possible mechanism by which amlodipine inhibits the onset of cardiovascular events in patients with atherosclerosis.

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