Periadventitial Inducible Nitric Oxide Synthase Expression and Intimal Thickening

Guido R.Y. De Meyer, Mark M. Kockx, Kristel M. Cromheeke, Cheikh I. Seye, Arnold G. Herman, Hidde Bult

Abstract—Positioning a silicone collar around the rabbit carotid artery induces a smooth muscle cell–rich intimal thickening. We investigated the localization of inducible nitric oxide synthase (iNOS) during thickening of the intima, the effect of iNOS inhibition on intimal thickness, and the effect of oxidized LDL (ox-LDL) on iNOS expression in the vessel wall. Collars were positioned for 18 hours or for 3, 7, or 14 days. Arterial cross sections were immunostained for iNOS, including naïve, sham-operated, and carotid arteries in which ox-LDL had been infused locally for 14 days. Furthermore, collars were connected to osmotic minipumps for local delivery (5 µL·h⁻¹, 14 days, n=12) of saline or the iNOS inhibitor L-NAME-(1-iminoethyl)lysine-HCl (L-NIL, 10 mmol/L). In the adventitia and the periadventitial granulation tissue of collared arteries, iNOS-positive macrophages and T lymphocytes were present from 18 hours onward. The media and intima were negative for iNOS. Reverse transcription–polymerase chain reaction revealed iNOS mRNA in collared but not in sham-operated arteries. Local inhibition of iNOS doubled the intimal thickness and decreased nitrotyrosine staining. Ox-LDL–treated arteries, which had a thicker intima, showed a pronounced influx of macrophages and T lymphocytes in all layers of the vessel wall, accompanied by iNOS expression in a subpopulation of these cells. Our study indicates that iNOS was not induced in intimal thickenings predominantly consisting of smooth muscle cells. However, iNOS was expressed in (peri)adventitial tissue and counteracted the progression of intimal thickening. Ox-LDL treatment was accompanied by an abundant influx of iNOS-positive macrophages and T lymphocytes in the vessel, but this could not prevent the progression of intimal thickening. (Arterioscler Thromb Vasc Biol. 2000;20:1896-1902.)

Key Words: intima | oxidized LDL | nitric oxide | adventitia | local delivery

Evidence in animals and humans indicates that atherosclerosis and intimal thickening, evoked by mechanical injury of the media, lead to the expression of inducible nitric oxide synthase (iNOS) in smooth muscle cells (SMCs) or macrophages within the arterial wall. Verbeuren et al. provided functional evidence that nonendothelial NOS is induced in the aortas of hyperlipidemic rabbits. Further studies showed that in rabbits, iNOS was present in cholesterol-induced plaques in T lymphocytes, macrophages, and SMCs. Also in human plaques, iNOS was found in association with macrophages, SMCs, endothelial cells (ECs), and mesenchymal-appearing intimal cells but not in normal vessels. Furthermore, arterial SMCs in the neointima formed after a deendothelializing balloon injury to the rat carotid artery expressed iNOS. Also, balloon angioplasty led to the induction of NOS in non-ECs of the rabbit carotid artery.

Positioning a silicone collar around the carotid artery of rabbits induces thickening of the intima. The collar model has the advantage that substances can be infused locally in the space between the collar and the arterial wall. Collar-induced intimal thickening is possibly the consequence of a combination of both vascular injury and hindrance of transmural flow by the collar, leading to retention of cytokines/growth factors within the segment enclosed by the collar. Although expression of iNOS can be induced by several cytokines, studies of the contractile behavior of isolated arteries have failed to demonstrate iNOS activity in the intima or media 14 days after collar placement. In contrast, a recent immunohistochemical study suggested abundant expression of iNOS in the intima of collared arteries.

The aim of the present study was to investigate possible explanations for this clear-cut discrepancy. We studied whether iNOS is expressed after collar-induced intimal thickening, and if so, its localization (intima, media, and/or adventitia) and whether collar-induced intimal thickening is affected by local treatment with the selective iNOS inhibitor L-NAME-(1-iminoethyl)lysine-HCl (L-NIL). L-NIL is 1 of the most selective iNOS inhibitors that are currently available. It is 11-fold more selective for iNOS than for endothelial NOS (eNOS) and 5- to 28-fold more selective for iNOS than
for neuronal NOS. Finally, in a retrospective study, iNOS expression was examined in intimal thickenings containing many monocytes and T lymphocytes in frozen tissue sections obtained from a previous study in which oxidized LDL (ox-LDL) was applied locally on the vessel wall.16

**Methods**

**Collar Implantation**

Male New Zealand White rabbits (2.5 to 3 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV). Both carotid arteries were surgically exposed and dissected from the surrounding tissues. In a first experiment, a flexible silicone collar (inlet/outlet diameter 1.8 mm, MED-4211 silicone; Nusil Technology) was positioned around the left carotid artery.11 The contralateral carotid artery was sham-operated; ie, it was isolated from the surrounding connective tissue and vagus nerve and was exposed to a similar stretch as the collared artery. The collar was left in position for 18 hours, 3 days, 7 days, or 14 days. In a second experiment, a silicone collar was placed around each carotid artery. The space (~100 µL) between the inner collar wall and the outer wall of the left carotid artery was connected to an osmotic minipump (Alzet 2 ML2, Alza Corp) by means of a sterile polyethylene catheter (Intramedic PE60, Clay Adams polyethylene tubing; Becton Dickinson). The minipump was placed subdermally in the thoracic region and delivered either the vehicle (saline containing 2 µg/mL polymyxin B at a rate of 5 µL/h, n=12 rabbits) or 10 mmol/L L-NIL (Alexis Corp; 5 µL/h, n=12 rabbits) continuously and locally to the carotid arteries for 14 days. Although the solutions were prepared in a sterile manner, polymyxin B was present in all infusions to bind possible traces of lipopolysaccharide.16,17 The collar around the right carotid artery was not connected to an osmotic minipump. All rabbits were fed a normal diet and had ad libitum access to tap water.

In a third, retrospective study, the frozen material obtained after perivascular infusion of ox-LDL for 2 weeks16 was stained for the presence of iNOS.

**Preparation of Ox-LDL**

LDL (Sigma, 4 mg protein per mL of PBS) was incubated with 6.4 mmol/L CuCl₂ for 16 hours at 37°C. After stopping the reaction by addition of 200 mmol/L EDTA and cooling on ice (1 hour), the Cu²⁺ ions were removed by extensive dialysis (24 hours, 4°C) against EDTA-containing PBS, and the solution was concentrated and steril-filtered as described.16

**Blood Pressure Measurements**

Blood pressure was measured 1 day before the rabbits were killed by using a Statham P2310 pressure transducer connected to an HP 88058 carrier amplifier. For this purpose, the skin of the ear of the rabbits was disinfected and sprayed with lidocaine (10% solution, Astra Nobelpharma), and a 22-gauge cannula was inserted into the central ear artery under translumination.

**Immunohistochemistry**

After 18 hours or 3, 7, or 14 days, the rabbits were humanely killed with an overdose of sodium pentobarbital, and 2 segments (2 to 3 mm) were cut from both the collar-treated zone of the carotid artery and the zone proximal to the collar (ie, sham-operated zone). One collagen and one sham-operated segment were fixed in 4% formalin for 24 hours. Then the carotid segments were dehydrated before being embedded in paraffin. A second segment (from both the collared and sham-operated zone) was immediately embedded in OCT (Tissue-Tek), frozen with LN₂, and used for frozen sections. In addition, in the 18-hour and 14-day groups, a third segment (from both collared and sham-operated vessels) was snap-frozen in LN₂ and used for reverse transcription–polymerase chain reaction (RT-PCR).

Staining for iNOS was done on both formalin-fixed and frozen sections. As primary antibodies, both a rabbit polyclonal (1:1000, Transduction Laboratories) and a mouse monoclonal (1:500, Transduction Laboratories) antibody were used. The secondary antibodies were, respectively, goat anti-rabbit (1:200, Vector Laboratories) and horse anti-mouse (1:200, Vector Laboratories). In addition, naïve and sham-operated carotid arteries and the carotid artery specimens obtained in a previous experiment, in which ox-LDL (oxidized with Cu²⁺ at 7 µg/h) had been infused locally into the interior of the collar for 14 days,16 were stained for iNOS. The primary antibodies were carefully titrated, and with every series of stainings, both negative (naïve carotid artery and omission of the primary antibody) and positive (granulation tissue after collaring and arteries from cholesterol-fed rabbits) controls were included. To confirm the in vivo activity of iNOS and the effect of L-NIL on NO production, we performed an immunohistochemical stain for nitrotyrosine (Upstate Biotechnology, Inc).19

Immunohistochemical detection of SMCs (α-SMC actin, clone 1A4; Sigma, 1:3 000 000) was done on both formalin-fixed and frozen preparations and of macrophages (CD68, clone EBM11; Dako, 1:1000) and T lymphocytes (CD43, clone L11/135; Dako, 1:300) on frozen preparations by using specific monoclonal antibodies. The antibody to α-SMC actin was detected by an indirect peroxidase antibody conjugate technique. The sections were incubated with a goat anti-mouse peroxidase antibody (Jackson) for 45 minutes. The antibodies to CD68, CD43, nitrotyrosine, and iNOS were detected with the ABC technique (avidin and biotinylated horseradish peroxidase macromolecular complex; Vectastain ABC kit, Vector Laboratories). 3-Amino-9-ethylcarbazole (AEC) was used as a chromogen.

In addition, double-labeling immunohistochemistry was performed to detect colocalizations of iNOS and CD68, iNOS and α-SMC actin, and iNOS and CD43. The antibodies were detected by using the Vectastain ABC kit. For iNOS, a peroxidase-coupled complex was used, and diaminobenzidine (DAB, Sigma) served as a chromogen. For the other antibodies, an alkaline phosphatase–coupled complex was used with fast blue BB (Sigma) as the chromogen.

**RNA Extraction and RT-PCR Analysis**

Total RNA was isolated by the guanidinium isothiocyanate and phenol/chloroform extraction method.20 RT-PCR was performed with an automatic thermal cycler (Mastercycler gradient, Eppendorf) and the ThermoScript 2-step RT-PCR system (Life Technologies). After denaturation of RNA and primer at 65°C for 5 minutes, reverse transcription was performed at 50°C for 60 minutes, followed by 85°C for 5 minutes. The final concentrations in the cDNA synthesis mixture (20 µL) were as follows: 3 µg total RNA; 2.5 mmol/L oligo(dT)20 primer; 5 mmol/L DTT; 40 U RNase inhibitor; 1 mmol/L dNTP mix; and 15 U Thermoscript reverse transcriptase. For the PCR step, the following specific primers were used: (1) rabbit iNOS mRNA sense (5′-GCT ACA CTT CCA ACG CAA CAT-3′; GenBank accession No. OCUS5094, positions 203 to 223) and rabbit iNOS mRNA antisense (5′-AAT CCA CTA GCT GCC CAA A-3′; GenBank accession No. OCUS5094, positions 457 to 493). Rabbit β-actin mRNA sense (5′-CGC GCA CCA GGG CGT-3′; GenBank accession No. X60733, positions 189 to 203) and rabbit β-actin mRNA antisense (5′-ATG GCC GCC GTG TGG AAC-3′; GenBank accession No. X60733, positions 453 to 470) primers were used as controls.21 The final concentrations in the PCR mixture (50 µL) were 1.82 mmol/L MgCl₂; 0.2 mmol/L dNTP mix; 0.2 mmol/L each sense and antisense primers; 2 U Platinum Taq DNA polymerase; and 2 µL cDNA preparation. The thermocycling parameters were as follows: denaturation of the template at 94°C for 2 minutes; 35 cycles consisting of incubations at 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 30 seconds; followed by a prolonged elongation time of 10 minutes at 72°C. Products were analyzed by agarose (2%) gel electrophoresis and visualized by ethidium bromide under UV light.

**Intimal Thickness**

The thickness of intima and media was measured using PC Image Color software (Foster Findlay) by an observer who was unaware of the treatment. From each artery 2 sections were analyzed and averaged, after measuring intimal and medial thicknesses at 20 random sites per section covering the whole ring.
Immunohistochemistry of iNOS

To reproduce a previously described experiment, we first used a rabbit polyclonal antiserum to iNOS on both formalin-fixed and frozen sections. To visualize this primary antibody, a donkey anti-rabbit IgG-peroxidase was used. There was strong immunoreactivity in the intima (Figure 1A). However, omitting the primary antibody (ie, using the secondary antibody alone) led to identical results (Figure 1B). This finding had not been mentioned previously. Sham-operated arteries were negative. The signal for nitrotyrosine was significantly decreased or even completely absent in the periadventitia (Figure 2S).

Statistical Analysis

All data are expressed as mean±SEM; n refers to the number of rabbits. Differences between the collared segment with and without infusion were evaluated by Wilcoxon signed-rank test with a significance level of 0.05.

Results

Immunohistochemistry of iNOS

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Therefore, we subsequently used a monoclonal antibody to iNOS on frozen sections from naïve, sham-operated, and collared carotid arteries. In naïve carotid arteries (Figure 2A) and sham-operated arteries (Figure 2B), iNOS was not detectable. After collaring of the carotid artery for 18 hours (Figure 2C) and 3 days (Figure 2D), there was a faint signal of iNOS immunoreactivity in the adventitia. After 7 days, immunoreactivity for iNOS was very clearly demonstrable in the adventitia and the periadventitial granulation tissue (Figure 2E). This staining pattern was similar after 14 days’ collaring but was even more pronounced (Figures 2F through 2H). Immunoreactivity for iNOS in the intima was completely absent (Figure 2G). Omitting the primary antibody resulted in the complete loss of immunoreactivity (Figure 2I).

The immunoreactivity of iNOS after L-NIL treatment was similar to that in the segments without L-NIL treatment (results not shown).

Double-labeling immunohistochemistry revealed colocalization with CD68 (Figure 2J) and CD43 (Figure 2L), indicating that iNOS was present in macrophages and T lymphocytes, respectively. In contrast, iNOS was not present in α-SMC actin–positive cells (Figure 2K).

In material obtained after local administration of ox-LDL, there was an abundant influx of monocytes and T lymphocytes into the vessel wall, and intimal hyperplasia was more pronounced. In this case, iNOS was present, not only in the periadventitia and adventitia but also in the media and the thick intima (Figures 2M and 2N). iNOS was localized in macrophages and T lymphocytes but not in SMCs (Figures 2O through 2Q). There was only a faint staining for iNOS in the ECs. The periadventitial staining of iNOS was similar in arteries with or without ox-LDL treatment.

Immunohistochemistry of Nitrotyrosine

In the saline-treated collared segments, nitrotyrosine was detected in the periadventitia (Figure 2R), which fits with the presence of iNOS in macrophages and T lymphocytes in the periadventitial granulation tissue after collaring. In contrast, after local application of the iNOS inhibitor L-NIL (vide infra), the signal for nitrotyrosine was significantly decreased or even completely absent in the periadventitia (Figure 2S).

RT-PCR of iNOS mRNA

We studied the presence of iNOS mRNA in sham-operated and collared arteries at both an early (18 hours) and a late (14 days) time point. iNOS mRNA was detected in arteries that had been collared for 14 days as the expected 291-bp amplified product. Omitting either the RNA or the reverse transcription step did not result in a band on the gel (data not shown). Sham-operated arteries were negative. The signal for β-actin mRNA (253 bp), which was used as an internal control, did not differ between the sham-operated and collared arteries (Figure 3). Eighteen hours after collaring, iNOS mRNA could not be detected (data not shown).

Effect of the iNOS Inhibitor L-NIL on Collar-Induced Intimal Thickening: Stability of the Inhibitory Capacity of L-NIL

To determine the stability of the inhibitory capacity of L-NIL, its effect on nitrite production of stimulated (lipopolysaccharide plus interferon-γ) J774 macrophages in culture was determined. There was no difference in the inhibitory capacity between a fresh solution of L-NIL and a solution that had been stored in osmotic minipumps placed in an incubator at 37°C for 14 days (data not shown).

Body Weight and Blood Pressure

There was no significant difference in body weight among the 3 treatment groups: 2.65±0.13 kg in the saline group; 2.68±0.15 kg in the L-NIL group at the start of the experiment; and 2.95±0.17 and 2.92±0.14 kg for the respective groups after 14 days of treatment. Two weeks of local treatment with L-NIL did not alter mean arterial blood pressure: 86±3 mm Hg in the saline group and 84±3 mm Hg in the L-NIL group.

Thickness of the Intima

Sham-operated segments proximal to the collar did not develop intimal thickening. After positioning a silicone collar around the carotid artery, intimal thickening was induced. Local delivery of saline did not increase intimal thickness compared with that in the contralateral artery, which had been surrounded by a collar not connected to a minipump (Figure 4). Local delivery of L-NIL doubled intimal thickening compared with the contralateral collared artery (Figure 4). Infusion of saline induced a small increase in medial thickness (132±4 versus 110±6 μm; P<0.05, n=12 arteries,
measured in duplicate). L-NIL had no effect on the thickness of the media.

**Discussion**

Annoying problems with the specificity of iNOS immunostains have recently been reported. The main conclusion was that it is essential to include appropriate controls for the species studied, and if possible, to confirm the immuno-staining by another technique such as RT-PCR. Hence, we carefully controlled our iNOS immunostains by using appropriate negative (naive rabbit carotid artery and omission of the primary antibody) and positive controls as well as RT-PCR.

Previously, the presence of iNOS in the intimal thickening induced by a periarterial collar in the rabbit carotid artery had been reported. In that study, a rabbit polyclonal iNOS antibody (primary antibody) and a donkey anti-rabbit IgG-peroxidase (secondary antibody) were used. However, we found that omitting the primary antibody resulted in complete loss of immunoreactivity. J through L. Determination of cell type in which iNOS is expressed in rabbit carotid arteries that had been collared for 14 days without ox-LDL treatment. Bars=50 μm. Brown (DAB) indicates iNOS; blue (fast blue BB) indicates CD68 (I), α-SMC actin (J), and CD43 (K). Nuclei were not stained. Cells that showed colocalization are indicated by arrow. J, Double-labeling immunostain for iNOS (brown) and CD68 (blue) in periadventitial granulation tissue, demonstrating colocalization (arrow) of iNOS and macrophages. K, Double-labeling immunostain for iNOS (brown) and α-SMC actin (blue) in periadventitial granulation tissue. α-SMC actin was absent, indicating that periadventitial iNOS expression was not present in SMCs. L, Double-labeling immunostain for iNOS (brown) and CD43 (blue) in periadventitial granulation tissue, demonstrating colocalization (arrow) of iNOS and T lymphocytes. M through S. Nitrotyrosine immunostain of rabbit carotid artery 14 days after collaring. Bars=50 μm. R, In saline-treated collared segments, nitrotyrosine (arrowheads) was present in periadventitia. S, In collared segments that had been locally treated with iNOS inhibitor L-NIL, signal for nitrotyrosine had significantly decreased or was completely absent in periadventitia.
the absence of the primary antibody, the immunoreactivity in the intima can be explained by reaction of the secondary antibody with IgG that has infiltrated into the intima of the collared but not the sham-operated arteries. Indeed, a drastic increase in the permeability of collared arteries for macromolecules such as fibrinogen has been reported, although the EC layer remains present in this model.

Furthermore, in that study, it had also been reported that carotid arteries that had been collared for 7 days, but not control arteries, produced an increase in resting tone after addition of N'G-nitro-L-arginine (10−5 mol/L), suggesting a higher basal release of NO in collared arterial segments than in controls. This confirmed our previous study, which demonstrated that N'G-nitro-L-arginine (2×10−5 mol/L) increased the contraction to KCl in collared arteries. However, this effect was seen only when ECs were present. The discrepancy between these 2 studies may be explained not only by the absence or presence of ECs but also by the differences in the presence of remnants of perivascular granulation tissue on the ring segments in the organ chambers.

By using a monoclonal antibody to iNOS on frozen sections of collared arteries, we demonstrated that iNOS was present in the adventitia and the perivascular granulation tissue surrounding collared arteries, which became very pronounced after 7 and 14 days. Double-labeling immunohistochemistry revealed the presence of iNOS in macrophages and T lymphocytes. However, we failed to detect iNOS immunoreactivity in the intima after 18 hours, 3 days, 7 days, or 14 days of collaring.

Taken together, our study indicates that in collar-induced intimal thickening, which predominantly consists of SMCs, iNOS is induced in the (peri)adventitial tissue. Moreover, the immunostain for nitrotyrosine, which is considered a footprint of the formation of peroxynitrite, proved that the iNOS protein displayed enzyme activity. The intimal or medial SMCs were not the main iNOS-expressing cell type in the injured artery, in contrast to findings by others in the balloon-denuded rat carotid artery. Because various cytokines such as interleukins-1 and -2 have been shown to be powerful stimulators of iNOS gene expression, the induction of iNOS in the (peri)adventitia may be caused by the retention of cytokines in the collared region.

In addition, we showed that the presence of iNOS in the adventitia and perivascular granulation tissue has functional consequences: blocking the (peri)adventitial iNOS strongly reduced the formation of nitrotyrosine residues and doubled the intimal thickness. The observation that the modest nitrotyrosine formation in ECs was not affected by L-NIL infusion points to selective inhibition of iNOS compared with eNOS. Therefore, NO formed by (peri)adventitial iNOS may be, at least in part, responsible for the fact that the thickening of the intima induced by the collar does not increase further after 14 days, ie, when iNOS expression became very pronounced. This fits with the concept that the adventitial response to arterial injury may be an important determinant of restenosis by influencing intimal thickening as well as remodeling.

Fourteen days of local treatment with L-NIL had no effect on the mean arterial blood pressure. This is not surprising, because the drug was infused locally in relatively small amounts. Even if all the material had reached the systemic circulation, the dose (6×10−8 g/kg body weight per minute) was still 10 000 times less than the ED50 of L-NIL for elevation of blood pressure. The immunoreactivity of iNOS after L-NIL treatment was similar to the segments without L-NIL treatment. Also, in a model of acute peritonitis in rats, L-NIL dramatically reduced NO levels without significantly affecting iNOS protein levels. Furthermore, L-NIL inhibits cytokine-induced NO production without affecting iNOS mRNA.

Our findings indicate that local inhibition of intimal thickening may be explained by interaction with iNOS-producing (peri)adventitial cells. We and others previously reported that supplementation with an NO donor reduced collar-induced intimal thickening. Furthermore, eNOS gene transfer as well as iNOS gene transfer inhibited intimal hyperplasia. NO generated from iNOS may prevent intimal thickening by inhibiting mitosis or migration of vascular SMCs, maintenance of the endothelial barrier function, or stimulation of EC regeneration. The latter possibility seems less likely. Because early detachment of ECs is very limited in the collar model, EC loss is not related to intimal hyperplasia, and full regeneration is already complete at 24 hours, ie, at a time when iNOS mRNA and protein were not yet expressed.

**Figure 3.** RT-PCR of iNOS mRNA. In arteries that had been collared for 14 days, iNOS mRNA was detected as the expected 291-bp amplified product (lane 2). Omitting either the RNA or reverse transcription step did not result in a band on the gel. Sham-operated arteries were negative (lane 1). Signal for β-actin mRNA (253 bp), which was used as a control, did not differ between sham-operated and collared arteries (lanes 3 and 4). M indicates molecular-weight marker (50-bp DNA ladder). Data are representative of 3 rabbits.

**Figure 4.** Intimal thickening in rabbit carotid artery after 14 days of collaring. Right carotid artery was surrounded by a collar that was not connected to an osmotic minipump ("collar without infusion"). Left carotid artery was also collared, but this collar was connected to an osmotic minipump ("collar with infusion") for local treatment with either saline or L-NIL for 14 days. Values are mean±SEM; n=11 or 12 arteries. **P<0.05 with L-NIL vs without L-NIL.**
Despite the observation that local ox-LDL treatment was accompanied by an abundant influx of iNOS-positive macrophages and T lymphocytes in the vessel, intimal thickness was increased. Several explanations are conceivable for this observation. First, the increased iNOS expression may lead to local levels of NO that may become detrimental. Second, the stimuli for intimal thickening evoked by ox-LDL may be too strong to be overcome by NO produced from iNOS. Third, the intimal thickening induced by collagen alone was mainly composed of α-SMC actin–immunoreactive cells, in contrast with the intimal thickening evoked by ox-LDL exposure, which showed an inhomogeneous expression of α-SMC actin but produced large amounts of collagen. Possibly, NO generated from iNOS has only an inhibitory effect on SMC proliferation and/or migration but not on collagen production. In summary, in collagen-induced intimal thickening, which predominantly consists of SMCs, iNOS is induced in (peri-)adventitial tissue but not in the intima. Blocking iNOS in the periadventitia doubles intimal thickness. Hence, we have presented evidence that adventitial iNOS counteracts the progression of intimal thickening, which may be relevant in maintaining arterial patency. In contrast, in intimal thickening, in which many inflammatory cells were elicited by local application of ox-LDL, the expression of iNOS was clearly detectable in the intima, media, adventitia, and periadventitia. Under these conditions, iNOS expression could not prevent progression of intimal thickening.

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References


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