Hypercholesterolemia Enhances Macrophage Recruitment and Dysfunction of Regenerated Endothelium After Balloon Injury of the Rabbit Iliac Artery

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Background. We studied the effects on and possible interaction of balloon denudation and hypercholesterolemia on large arteries in the rabbit with special regard to structure and vascular reactivity.

Methods and Results. New Zealand White rabbits fed a 1% cholesterol diet or a standard diet for 14 weeks underwent balloon denudation of the left iliac artery 4 weeks before death. Both the balloon-injured and the control iliac arteries were harvested for in vitro studies of vascular reactivity, for immunohistochemical staining with monoclonal antibodies directed at smooth muscle cells and macrophages, and for scanning electron microscopy. Balloon injury caused intimal smooth muscle proliferation with little macrophage infiltration and was followed by recovery of endothelium-dependent vasodilator function within 4 weeks. Hypercholesterolemia caused macrophage-rich lesions confined to the intima with moderate impairment of endothelial vasodilator function. Balloon injury in the setting of hypercholesterolemia caused intimal smooth muscle cell proliferation and intense macrophage infiltration throughout the arterial wall and severe impairment of endothelial vasodilator function. Scanning electron microscopy confirmed regrowth of the endothelium in all balloon-injured vessels. In the balloon-injured arteries of hypercholesterolemic animals, the regenerated endothelium exhibited areas of atypical morphology not seen after balloon injury or hypercholesterolemia alone.

Conclusions. The present study shows that balloon injury, hypercholesterolemia, and their combination cause distinct lesions and functional disturbances. An arterial balloon injury in the setting of hypercholesterolemia produces a diffuse inflammatory response that is accompanied by a sustained impairment of endothelial function and a marked proliferative response. (Circulation 1991;84:755-767)

Intimal thickening in atherosclerosis is the result of proliferation of smooth muscle cells that have migrated from the media as well as the infiltration of circulating monocytes and their transformation into foam cells.1 This process is associated with widespread disturbances in arterial function, including impairment in endothelium-dependent relaxation.2-10 Studies that mimic certain aspects of atherosclerosis in patients have been performed in animals. Intimal thickening resembling atherosclerosis with cellular infiltration, proliferation, and transformation into foam cells has been induced by administration of a high cholesterol diet,1 by mechanical (e.g., balloon) injury to the arterial wall,11 or by a combination of the two methods.12 These injurious stimuli have their clinical counterparts in patients with hyperlipidemia, in patients whose arteries have been damaged by mechanical means (e.g., balloon angioplasty), or in patients with a combination of these processes. The purpose of the present study was to

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investigate how hypercholesterolemia and balloon injury interact in eliciting cellular responses and causing impairment in endothelial (vasodilator) function.

Methods

Twenty-five male New Zealand White rabbits weighing 2.8–3.9 kg were studied. The rabbits were fed either a 1% cholesterol diet (Purina Chow, St. Louis; n=13) or a standard diet (n=12) for 14 weeks. All rabbits underwent balloon injury of the left iliac artery 10 weeks after initiation of the diet. Four weeks after this procedure, all of the rabbits were killed. Vascular responsiveness was examined in vitro in the left (injured) and right (control) iliac arteries of seven animals of each dietary group. Thus, the iliac arteries were examined under one of four experimental conditions: group 1) no balloon injury and a normal diet, group 2) balloon injury and a normal diet, group 3) no balloon injury and a high cholesterol diet, or group 4) balloon injury and a high cholesterol diet. Iliac arteries harvested from 11 animals were studied for evidence of histological abnormalities using scanning electron microscopy (n=6) or immunohistochemical staining (n=5). Vascular reactivity and structure were assessed concurrently in arteries from the four groups; the results of the vascular reactivity experiments from group 2 (balloon injury and a normal diet) were included in a previous report.11

Balloon Injury

The procedure for inducing a balloon injury of a rabbit iliac artery has been described in detail previously.11 In brief, the animals were anesthetized with thiopental (10 mg/kg) followed by sodium pentobarbital (20 mg/kg) given intravenously. A 2.5-mm balloon angioplasty catheter (Advanced Cardiovascular Systems, Temecula, Calif.) was introduced through the right carotid artery and advanced over a 0.014-in. guidewire under fluoroscopic guidance into the left iliac artery. The balloon was inflated in the distal portion of the external iliac artery to 8 atm for three 30-second intervals. The inflated balloon was then pulled back 1 cm, and an additional three inflations were performed. This procedure has been shown to cause complete endothelial denudation over a length of approximately 2 cm without disruption of the internal elastic lamina.11

Studies of Endothelial Vasodilator Function

Four weeks after the balloon angioplasty, the animals were killed with an overdose of pentobarbital, and the iliac arteries were excised and immersed in cold physiological saline solution. Vessels were cleaned of adherent tissue, and 5-mm-long rings were cut, mounted horizontally in organ chambers filled with physiological saline solution at 37°C, and continuously oxygenated (95% O₂–5% CO₂). Two pairs of rings from the proximal and distal portions of each iliac artery (balloon-injured and control arteries) were studied simultaneously. Iso- metric tension recordings were obtained with a linear force transducer (FT03, Grass Instruments, Quincy, Mass.) and polygraph (model 79D, Grass). The optimal length–tension relation was determined in each ring by progressively stretching it and measuring the response to 10⁻⁹ M norepinephrine. Subsequently, the rings were exposed to increasing concentrations of norepinephrine (10⁻⁹ to 10⁻⁴ M) to determine the norepinephrine concentration inducing the half-maximal response (E₅₀). Next, the vascular ring was washed repeatedly with fresh physiological saline solution until tension returned to the previous baseline value. Indomethacin (10⁻⁵ M) was added 30 minutes before any study of endothelium-dependent relaxation. To study the effects of vasodilator agents (e.g., acetylcholine, calcium ionophore A23187, nitroprusside), the vessel was first contracted by the E₅₀ dose of norepinephrine. When a stable contraction was obtained, the tissue was exposed to increasing concentrations of the vasodilator agent in a cumulative fashion.

In addition, some tissues were exposed to increasing concentrations of the vasoconstrictor serotonin (10⁻⁸ to 10⁻⁴ M in half-log unit increments).

Histological Studies

Light microscopy. All rings studied in the organ chamber were subsequently fixed in 1.25% glutaraldehyde with 0.1 M cacodylate buffer (pH 7.4), embedded in paraffin, and stained with van Gieson elastic stain or hematoxylin and eosin. Intimal and medial thicknesses were measured in three cross sections per ring, as previously described.11 This morphometric analysis was performed in vascular rings previously studied in the organ chamber. The advantage of this approach is that physiological and histomorphometric measurements are made in the same tissue. However, the histomorphometric measurements are only semiquantitative because the tissues were not fixed under physiological distending pressures.

Scanning electron microscopy. In six rabbits (n=3 from each dietary group) 4 weeks after angioplasty, the iliac arteries were fixed by antegrade perfusion with 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.4) at a pressure of 100 mm Hg through a cannula inserted into the abdominal aorta. After 15 minutes of perfusion with the fixative, both iliac arteries were excised, and fixation was continued for 24 hours at 4°C. The artery segments were then critically point dried, longitudinally bisected, sputter-coated with gold and palladium, and examined in an AMR 1400 scanning electron microscope.

Immunohistochemistry. Immunohistochemical staining was performed on 6-μm frozen sections, fixed at −20°C for 5 minutes in 1:1 acetone:methanol. Monoclonal antibodies CGA7 and RAM11, kindly provided by Dr. A. Gown, University of Washington, were used to identify in serial sections vascular smooth muscle cells and macrophages, respectively. Sections were incubated (22°C) in succes-
sion with monoclonal antibodies (2 hours), biotinilated horse anti-murine IgG (1 hour), and avidin-biotin peroxidase complexes (45 minutes) (Vector Labs, Inc., Burlingame, Calif.). Peroxidase was visualized with 3-amino-9-ethylcarbazole (Sigma Chemical, St. Louis), and sections were counterstained with Gill's hematoxylin.

Serum Cholesterol Determinations

Blood was collected from all rabbits at the time of balloon angioplasty. Total serum cholesterol was measured with an automatic analyzer (SMAC, Technical Corp., Tarrytown, N.Y.).

Drugs

The drugs used in the physiological studies were acetylcholine chloride, calcium ionophore (A23187), indomethacin, norepinephrine bitartrate, and sodium nitroprusside (Sigma). All drugs were prepared daily with distilled water, except for A23187, which was dissolved in dimethylsulfoxide (DMSO; Sigma). DMSO alone in concentrations of as much as 0.2% in the organ chamber did not cause a vascular response. The drugs were kept on ice during the experiment.

Statistical Analysis

Results are expressed as mean±SEM. To compare vasodilator responses, we determined the maximal response (expressed as percent relaxation of the norepinephrine-induced contraction) and the EC50 (concentration of drug inducing 50% relaxation of the norepinephrine-induced contraction; expressed as -log M) for each concentration–response curve. EC50 rather than EC50 was chosen to compare vasodilator responses because the relaxation of the norepinephrine-induced contraction did not reach 50% for some concentration–response curves. For contractions, the maximal response (in grams of tension) and the EC50 concentration of drug inducing the half-maximal contraction were determined. Differences between group means were evaluated by Student's t test for unpaired data (for cholesterol concentrations) or by a one-way analysis of variance whenever multiple comparisons were made (e.g., organ chamber data, morphometric data) followed by Scheffe's test to identify these differences. The correlation between morphometric measurements of intimal thickness and relaxation was analyzed by linear regression. Statistical significance was set at a probability value of less than 0.05.

Results

Serum Cholesterol Levels

At the time of balloon injury, after 10 weeks of feeding, mean serum cholesterol concentration was 1,820±206 mg/dl (range, 1,100–2,740 mg/dl) in the cholesterol-fed rabbits and 65±11 mg/dl (range, 30–122 mg/dl) in the standard diet group (p<0.001).

Functional Studies

In control arteries of normocholesterolemic animals, acetylcholine caused complete relaxation (Figure 1 and Table 1). There was no significant impairment of endothelium-dependent relaxation in response to either acetylcholine or A23187 4 weeks after balloon injury alone. Hypercholesterolemia alone induced a mild impairment in the response of the artery to acetylcholine evidenced by a slight rightward shift in the concentration–response curve to acetylcholine, but the maximal relaxation was not significantly different from control (Figure 1 and Table 1). In contrast, the combination of balloon injury and hypercholesterolemia resulted in marked reduction of acetylcholine-induced relaxation compared with the controls or either intervention alone (Figure 1 and Table 1). A similar pattern of synergistic impairment of endothelium-dependent relaxation by hypercholesterolemia and balloon injury was observed in response to the calcium ionophore A23187, the actions of which do not require specific membrane receptors (Figure 2 and Table 1). In contrast, relaxation to sodium nitroprusside was normal in all arteries studied. There was evidence of enhanced sensitivity to the nitrovasodilator in the arteries exposed to the combined intervention evidenced by a leftward shift in the dose–response relation (Figure 3 and Table 1).
TABLE 1. Endothelium-Dependent and -Independent Vasodilator Responses of Rabbit Iliac Arteries After Exposure to Hypercholesterolemia, Balloon Injury, or Both Interventions

<table>
<thead>
<tr>
<th>EC50 (−log M)</th>
<th>Control (n=7)</th>
<th>Balloon (n=7)</th>
<th>Cholesterol (n=7)</th>
<th>Balloon+Cholesterol (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>7.3±0.1</td>
<td>7.1±0.1</td>
<td>6.6±0.1</td>
<td>6.2±0.3*</td>
</tr>
<tr>
<td>A23187</td>
<td>7.1±0.1</td>
<td>6.4±0.07†</td>
<td>6.8±0.1</td>
<td>6.3±0.1‡</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td>5.9±0.3</td>
<td>6.3±0.3</td>
<td>6.6±0.2</td>
<td>7.2±0.1§</td>
</tr>
</tbody>
</table>

Maximal relaxation (%)

| Acetylcholine | 99±0.6       | 96±2          | 82±9             | 45±9||                  |
| A23187       | 95±3         | 86±7          | 78±6             | 38±8||                  |
| Nitroprusside| 89±11        | 92±8          | 100±0.5          | 99±0.9                   |

Data for maximal relaxation are expressed as percentage of initial tension in response to norepinephrine. Values are given as mean±SEM.

*p<0.05 vs. control, balloon.  
†p<0.05 vs. cholesterol.  
‡p<0.05 vs. control, cholesterol.  
§p<0.05 vs. control.  
||p<0.05 vs. control, balloon + cholesterol.  
Level of significance was tested by analysis of variance followed by Scheffe's test.

Contractile responses are summarized in Table 2. Neither intervention alone affected the sensitivity of these arteries to norepinephrine or serotonin (no change in EC50 or maximal response). The combination of the two interventions did not affect the sensitivity (EC50) of these vessels to norepinephrine or serotonin but did attenuate the maximal contraction.

Morphological Studies

Light microscopic cross sections. Vessels injured by balloon angioplasty or exposed to the cholesterol diet exhibited moderate and focal intimal proliferation (Figure 4). The internal elastic lamina of these arteries appeared generally intact. Balloon-injured arteries of hypercholesterolemic rabbits exhibited marked intimal proliferation that exceeded that after balloon injury or hypercholesterolemia alone (Figure 4). Foam cells were abundant in these lesions and were present in both the intima and the media of the arteries (data not shown).

Immunohistochemistry. The intima of control iliac arteries consisted of a monolayer of flat endothelial cells in close apposition to the internal elastic lamina. There was no evidence of intimal smooth muscle or monocyte/macrophage infiltration (data not shown).

Four weeks after balloon angioplasty in normocholesterolemic rabbits, a significant portion of the iliac luminal surface was involved by intimal lesions composed predominantly of elongated smooth muscle cells (Figure 5). Hypercholesterolemia alone induced focal foam cell–rich intimal lesions. The majority of these foam cells stained with RAM11 monoclonal antibody, which is indicative of their monocyte/macrophage origin (Figure 6). The media of both balloon-injured normocholesterolemic and uninjured hypercholesterolemic iliac arteries was essentially normal except for occasional small foci of foamy macrophages. Arteries exposed to balloon angioplasty in the setting of hypercholesterolemia contained extensive near-circumferential intimal lesions comprising abundant smooth muscle cells and, to a lesser degree, foamy macrophages. The media and adventitia of these vessels were densely infil-
trated by monocyte/macrophages with loss of medial elastic tissue (Figure 7).

*Scanning electron micrographs.* Scanning electron microscopy of the control iliac arteries revealed an intact lining with fusiform cells aligned in the direction of blood flow, which is typical of endothelium. Morphology of the luminal lining cells was also normal in arteries subjected to balloon injury or cholesterol diet alone (Figure 8A). In contrast, in arteries exposed to the combined intervention, the endothelium exhibited areas of distinct morphologic abnormality, with many polygonal-shaped endothelial cells of varying sizes, large interendothelial gaps, and foci of adherent leukocytes on the luminal surface (Figure 8B).

*Correlation of functional and morphological changes.* There was a strong inverse correlation between maximal relaxation to acetylcholine and the extent of intimal thickening after the combined intervention ($r = -0.88, p < 0.0001$; Figure 9).

**Discussion**

Results from the present study demonstrate that the three types of injury cause characteristic cellular lesions and functional disturbances in rabbit iliac artery. Balloon injury causes intimal smooth muscle accumulation, presumably the result of smooth muscle cell migration from the media and proliferation in the intima, without prominent monocyte/macrophage infiltration and normal endothelium-dependent vasodilator function within 4 weeks. Hypercholesterolemia causes foamy macrophage-rich lesions confined to the intima with moderate impairment of endothelial vasodilator function. A combined injury induced by balloon angioplasty in the setting of hypercholesterolemia causes both intimal smooth muscle cell proliferation and intense macrophage infiltration in the intima, media, and adventitia, with severe impairment of endothelial vasodilator function.

We have shown that changes in vascular reactivity after balloon injury depend on its severity and the time elapsed since the injury. After a "severe injury," induced by an oversized balloon approximately 30% larger than the luminal diameter, marked impairment in endothelial function persisted for 4 weeks or longer. This abnormal function was characterized by an atypical appearance of endothelial cells, which were irregular in size, columnar rather than flat, and not oriented in the direction of blood flow. A "moderate injury," induced with an

**Figure 3.** Concentration–response curves to sodium nitroprusside in rings from control iliac arteries compared with arterial rings 4 weeks after balloon angioplasty (B), rings from hypercholesterolemic rabbits (Chol), and rings from hypercholesterolemic rabbits 4 weeks after balloon angioplasty (B+Chol) (n=7 in each group). *p<0.05 vs. control. NE, norepinephrine.

**Figure 4.** Bar graphs of intimal/medial thickness ratios in rings from control iliac arteries compared with arterial rings 4 weeks after balloon angioplasty (B), rings from hypercholesterolemic rabbits (Chol), and rings from hypercholesterolemic rabbits 4 weeks after balloon angioplasty (B+Chol). Balloon-injured arteries of hypercholesterolemic rabbits exhibited marked intimal proliferation that exceeded that seen after balloon injury or hypercholesterolemia alone. *p<0.05 vs. all other groups.
FIGURE 5. Microphotographs showing immunohistochemical staining of iliac arteries of normocholesterolemic rabbits 4 weeks after balloon injury. Top panel: Stain for smooth muscle demonstrates predominance of smooth muscle cells in thickened intima and expected staining of media. Bottom panel: Serial section of same balloon-injured artery stained for macrophages shows only a few, scattered macrophages that are confined to the intima. Magnification bar, 100 μm. Arrow, internal elastic lamina.

FIGURE 6. Microphotographs (right) showing immunohistochemical staining of iliac arteries of hypercholesterolemic rabbits. Top panel: Stain for smooth muscle demonstrates only a few smooth muscle cells in focal lesions in intima and expected staining of media. Bottom panel: Serial section stained for macrophages shows that focal intimal lesions induced by hypercholesterolemia are predominantly composed of macrophages. Magnification bar, 100 μm. Arrow, internal elastic lamina.
angioplasty balloon of approximately the same size (2.5 mm) as the lumen of rabbit iliac arteries, caused a partial impairment in the vasodilator function of the regenerated endothelial cells at 2 weeks with return of normal function within 4 weeks. These data suggested that at 2 weeks, dysfunction of newly regenerated endothelial cells is potentially reversible. The present study, which used a moderate injury, extends these observations to show that this return of vasodilator function at 4 weeks in the normocholesterolemic setting is associated with restoration of normal endothelial morphology.

Modulation of Endothelial Vasodilator Function by Macrophages

In the present study, a moderate balloon injury in the setting of hypercholesterolemia resulted in macrophage infiltration throughout the arterial wall and was associated with severe, persistent impairment of endothelium-dependent relaxation despite complete endothelial regrowth. Endothelium-dependent relaxation has been shown to be impaired in both experimental and human atherosclerosis12-19 due to a diminished production or release of endothelium-derived relaxing factor (EDRF).13-15 In addition, when atherosclerosis is induced by a combination of high cholesterol diet and balloon injury, relaxation to exogenous EDRF may become impaired, consistent with the concept that under certain conditions an atherosclerotic artery can also present a functional barrier to nitric oxide (EDRF) diffusion.14 Although the factors responsible for the loss of endothelium-dependent relaxations in atherosclerosis have not been identified, the intense macrophage infiltrate could play a role by several different mechanisms. First, cytokines, products of macrophages, are known to modulate properties of endothelial cells.16 One such cytokine, tumor necrosis factor, has been found in abundant amounts in atherosclerotic plaques of humans17 and has been shown to act on the endothelial cell to inhibit endothelium-dependent relaxations to acetylcholine.18 Second, administration of L-arginine, the substrate for the endothelial production of EDRF, has been shown to restore endothelium-dependent responses in hypercholesterolemic rabbits.19 This finding has suggested that endothelial cells in atherosclerosis are depleted of L-arginine, probably because of a diminished uptake of this precursor.20 Macrophages could play a secondary role in this process by further depleting the arterial wall of L-arginine, either by degrading it via the enzyme arginase, which is induced during macrophage activation,20 or by converting arginine to macrophage-derived nitric oxide.21 Third, superoxide anion, a product of macrophages, can directly inactivate EDRF, thus potentially presenting a functional barrier to EDRF action.22,23 Last, macrophages can oxidize low density lipoproteins (LDL).24 Although unmodified LDL is capable of inhibiting endothelium-dependent relaxations,25-27 its oxidized form is particularly potent in this regard,28 and oxidized LDL has been isolated from arteries of rabbits and humans with hypercholesterolemia.29

Factors Relating Hypercholesterolemia to Macrophage Infiltration

The diffuse monocyte/macrophage infiltration after balloon injury of hypercholesterolemic arteries was an unexpected finding because in the process of removing endothelium, angioplasty caused only minimal injury to the media. In the normocholesterolemic animals exposed to an identical injury, the media appeared to be essentially normal, except for occasional disruptions in the internal elastic lamina and rare foci of macrophages. The impaired responses to contractile agonists that developed after balloon injury in hypercholesterolemic arteries provide functional evidence for the significance of morphological changes in the media. Because balloon injury alone did not cause appreciable medial damage, products of cell necrosis are not likely to be the principal mediators of the subsequent leukocyte infiltration. Several other mechanisms may be operative. First, hypercholesterolemia has been shown to promote monocyte chemotaxis and activation. Several researchers30-33 have isolated factors from atherosclerotic lesions in hypercholesterolemic animals that preferentially stimulate monocyte migration in a chemotactic assay. Second, intimal denudation with angioplasty may provide a setting whereby penetration of lipoproteins into the media can be enhanced and their metabolism in the media altered. These lipoproteins and their oxidized form could act as a chemoattractant for monocyte/macrophages, producing a focus of inflammation.34 Abundant lipids in the vessel wall are known to act as foci of inflammation in human atherosclerotic lesions.35 Last, the local generation of cytokines and induction of endothelial-leukocyte adhesion molecules, both in adventitial blood vessels and in regenerating luminal endothelium, may also contribute to monocyte/macrophage recruitment. Injured vascular cells, including smooth muscle cells and endothelial cells, can produce cytokines such as interleukin-1,36,37 as can infiltrating leukocytes. These mediators can induce a highly adhesive surface for monocyte/macrophages on endothelial cells that appears to be in part the result of surface expression of endothelial adhesion molecules.38 Recently, the expression of a monocyte-directed adhesion molecule localized to endothelium overlying arterial intimal lesions has been described in hypercholesterolemic rabbits.39

Correlation Between Intimal Thickness and the Degree of Impaired Response to Acetylcholine

After arterial injury in the hypercholesterolemic setting, the degree of impairment in endothelium-dependent relaxation correlated with the extent of intimal proliferation. It may be that the thickened intima formed a functional or physical barrier to EDRF that has to move by diffusion from the endothelium to act on the smooth muscle cells in
the media. EDRF has a short half-life\textsuperscript{39,40} that is reduced further by superoxide anion,\textsuperscript{22,23} a product of macrophages that were present in abundance throughout the vessel wall in this model. Alternatively, intimal thickness may simply be a marker of endothelial dysfunction. The vessels with the thickest intima may have the greatest degree of impairment in endothelial function, and this correlation may reflect only the severity of the disease process affecting the endothelium. The finding of a correlation between intimal thickness and impairment of endothelium-dependent relaxation has been reported in an atherosclerotic porcine model.\textsuperscript{41} In this model, sites of histamine-induced spasm were lo-
calized to areas of intimal thickening. The inference from our findings as well as from findings of previous studies is that intimal thickening is closely associated with the abnormal responsiveness of the arterial wall to a variety of endothelium-dependent vasoactive agents.

Role of Endothelial Dysfunction in Intimal Proliferation

In addition to regulating vasomotor tone, the normal endothelium inhibits vascular smooth muscle cell proliferation. This inhibition is mediated in part by the production of heparinlike molecules; recently, nitric oxide (EDRF), other nitrovasodilators, and cyclic GMP were found to inhibit vascular smooth muscle cell proliferation in vitro. Thus, it may be that the marked intimal proliferation observed after balloon injury in the hypercholesterolemic setting is a result in part of reduced release of the endogenous nitrovasodilator EDRF. Although the intimal thickening observed in the acute phase in this model may be principally related to endothelial denudation and platelet deposition as well as to acute arterial distension, the dysfunctional endothelium regenerating after angioplasty may contribute to an imbalance between growth-promoting and growth-inhibiting factors and allow the myointimal cell proliferation to continue unimpeded.

Augmented Vasodilation by Sodium Nitroprusside in Arteries With Endothelial Vasodilator Dysfunction

The reduced dilation of the balloon-injured arteries in the hypercholesterolemic setting in response to
the endothelium-dependent agents acetylcholine and calcium ionophore A23187 was not because of an inability of vascular smooth muscle to respond to EDRF (nitric oxide) because the response to sodium nitroprusside, an exogenous donor of nitric oxide, was not impaired and was slightly, yet significantly, enhanced. The vasodilator actions of exogenous nitrovasodilators may be augmented acutely by endothelial removal and chronically by diseases that damage the endothelial lining. EDRF and other nitrovasodilators produce relaxation predominantly by activation of guanylate cyclase in vascular smooth muscle. Available evidence suggests that basal formation of EDRF interferes with the relaxant effects to exogenous nitrovasodilators. The removal of this basal influence of EDRF appears to sensitize the guanylate cyclase enzyme to agents that act by the stimulation of this pathway.

**Clinical Implications**

In patients, hypercholesterolemia and arterial injuries that are acute (balloon angioplasty) or recurrent (hemodynamic stresses) are important processes that characterize the development of obstructive arterial disease. The present study shows that an arterial balloon injury, hypercholesterolemia, and their combination produce distinct lesions. An arterial injury in the setting of elevated serum cholesterol can result in diffuse macrophage infiltration, sustained impairment of endothelial function, and marked intimal proliferation. The results of the present study in rabbits have to be applied to the clinical setting with caution because of the marked degree of hypercholesterolemia. However, the direct relation of hyperlipidemia to the increased incidence of restenosis in patients undergoing balloon angioplasty has been established. In addition, a preliminary study has suggested that cholesterol-lowering therapy reduces the incidence of restenosis after coronary angioplasty.

The present study suggests possible mechanisms. Our data indicate that the normal inhibitory function of endothelium is lost when balloon angioplasty is performed in the hypercholesterolemic setting. The loss of this influence and the stimulatory influence of infiltrating macrophages, cells rich in growth-promoting factors, may explain the tendency of hypercholesterolemia to accentuate the restenosis process and atherogenesis. The appreciation of the interaction among hypercholesterolemia, endothelium, and macrophages should provide new avenues in the treatment of restenosis and atherogenesis.

**References**


**FIGURE 9.** Plot showing correlation of intimal medial thickness with maximal relaxation to acetylcholine (Ach) in arterial rings from hypercholesterolemic rabbits 4 weeks after angioplasty. Thickening of intima correlated inversely with maximal relaxation to Ach (r = −0.88, p = 0.0001).


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