Persistent Dysfunction of Regenerated Endothelium After Balloon Angioplasty of Rabbit Iliac Artery

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This study investigated the vasodilator function of endothelium that regenerated after balloon angioplasty and the relation of this function to the extent of vascular injury and to subsequent intimal proliferation. Balloon angioplasty was performed in the left iliac artery of 47 New Zealand White rabbits. Vascular responses were examined in vitro 2 and 4 weeks after a “severe” injury (3.0-mm balloon) or a “moderate” injury (2.5-mm balloon). Both degrees of balloon injury caused complete endothelial denudation. Endothelial regrowth 2 weeks after either injury was confirmed histologically. Although the regenerated cells had irregular sizes and polygonal shapes and lacked the typical alignment in the direction of blood flow, immunocytochemical staining for factor VIII–related antigen identified these cells as endothelium. To study the vasodilator function of regenerated endothelium, rings of balloon-injured and control (contralateral) iliac arteries were suspended in organ chambers for recording of isometric force. Endothelium-dependent relaxation of balloon-injured vessels to acetylcholine and to the calcium ionophore A23187 were reduced at 2 and at 4 weeks after severe injury. After moderate injury, endothelium-dependent relaxations to these agents were reduced at 2 weeks but had normalized by 4 weeks. Endothelium-independent relaxation to sodium nitroprusside, however, was preserved in all study groups. Morphometric analysis revealed an inverse correlation between the degree of intimal thickening and maximal relaxation to acetylcholine (r=0.45, p<0.01). Thus, there is a persistent attenuation of receptor- and nonreceptor-mediated endothelium-dependent relaxations after arterial injury. The regenerated cells have an altered morphological appearance, but staining for factor VIII–related antigen confirms their endothelial origin. The degree and duration of endothelial dysfunction depends on the severity of the initial injury and is related to the extent of intimal thickness. (Circulation 1990;81:1667–1679)

Normal endothelial function includes the formation of a semipermeable barrier that actively regulates coagulation, cell growth, and vasoreactivity.1–4 Endothelium-derived relaxing factor (EDRF), a substance released from endothelial cells in response to various chemical and physical stimuli,4 is a potent inhibitor of vascular smooth muscle tone and platelet aggregation.3–7 More recently, nitrovasodilators and cyclic GMP were observed to inhibit proliferation of vascular smooth muscle in vitro8; therefore, the endogenous nitrovasodilator EDRF probably also suppresses growth of vascular smooth muscle.

Experimentally, removal of the endothelium by balloon injury leads to acute platelet deposition,9,10 vasoconstriction,11 and if the injury is extensive, it may be followed by intimal smooth muscle cell proliferation.12,13 Healing of the injured intima involves migration and proliferation of endothelial cells from the edges of the lesion.14,15 Despite regrowth of the endothelium, however, its function may not return to normal. Aggregating platelets or
serotonin induce endothelium-dependent relaxations of the porcine coronary artery in vitro. After recovery from balloon injury, these endothelium-dependent
relaxations are impaired. Furthermore, despite regrowth of luminal lining cells (thought to represent 
regenerated endothelium), intimal proliferation of 
vascular smooth muscle progresses.

These observations indicate that the endothelium regenerating after balloon angioplasty may be dys-
functional with respect to its inhibition of vascular 
reactivity and growth. Therefore, the aims of the 
present study were to examine endothelial vasodilator 
function after different degrees of arterial injury and 
to test the hypothesis that the degree of vascular 
smooth muscle proliferation after balloon angioplasty 
is related to the severity of endothelial dysfunction.

Methods

Forty-seven male New Zealand White rabbits, 
weighing between 2.0 and 3.2 kg, were studied. The 
left iliac artery was chosen for angioplasty to allow 
use of the contralateral iliac artery as an uninjured 
control vessel. Vascular reactivity was examined in 
vitro 2–3 days (n = 3), 2 weeks (n = 12), and 4 weeks 
(n = 12) after angioplasty. Because of the known 
influence of balloon size on vessel damage, we 
exposed the vessels to different degrees of injury with 
a 2.5-mm (“ moderate” injury) or a 3.0-mm balloon 
(“severe” injury) and compared the effects after 2 
and 4 weeks (n = 6 for each group). All groups were 
weight matched at the time of angioplasty.

Angioplasty

The rabbits were anesthetized with thiopental (10 
mg/kg) followed by sodium pentobarbital (20 mg/kg) 
intravenously. The right carotid artery was exposed, 
and a coronary angioplasty catheter (Advanced Car-
diovascular Systems, Temecula, California) was 
passed over a steerable guide wire (0.014 in.) into the 
left iliac artery. Beginning at the distal portion of 
the external iliac artery, the contrast-filled balloon 
inflated three times to 8 atm for 30 seconds. After 
withdrawing the partially deflated (2–4 atm) balloon 
about 1 cm, we performed another set of three infla-
tions. In preliminary experiments, histological exami-
nation of arteries en face with Evans blue dye (0.5% in 
saline solution, 2 ml/kg i.v. injected 30 minutes after 
angioplasty) or silver nitrate staining demonstrated 
that this procedure resulted in complete removal of 
endothelium from an area of about 2 cm in length. 
Angiograms obtained in four animals showed a ratio 
of inflated balloon to luminal diameter of approxi-
mately 1.25 (moderate injury) and 1.5 (severe injury). 
Postdilatation angiograms revealed that only the 
larger balloon caused a persistent dilation of the vessel 
by about 30% of the initial diameter.

Histological Study

Examination of regenerated endothelium. Cross 
sections. To examine the tissue response to the 
balloon injury and the morphological features of 
regenerated endothelial cells, vascular rings studied 
in the organ chamber were subsequently fixed in 1.25% glutaraldehyde with 0.1 M cacodylate buffer 
(pH 7.4), embedded in epon, and stained with tolu-
idine blue. In addition, four rabbits (two moderate 
and two severe injuries) were killed for histological 
study only, and cross sections from the iliac arteries 
were prepared in the same manner.

Silver nitrate staining. Using in situ staining 
with silver nitrate, we examined endothelial re-
growth at 2 (n = 3) or 4 weeks (n = 4). Briefly, the 
abdominal aorta was cannulated immediately after 
death and then perfused sequentially with solutions 
of 5% dextrose, 0.25% AgNO3, 1% NH4Br, and 3% 
CoBr each for 1 minute, followed by 100 ml buffered 
formalin. The aorta and iliac arteries were excised, 
immersed in formalin for about 1 hour, and rinsed in 
cacodylate buffer. Control and injured iliac arteries 
were cut into rings of a 1-cm length, incised longitudi-
nally with iridectomy scissors, dehydrated, 
mounted flat on a glass slide with the endothelial 
surface up, and examined by light microscopy.

Scanning electron microscopy. In three animals, 
morphological features of the endothelium were 
examined by scanning electron microscopy 4 weeks 
after severe injury to the left iliac artery. The animals 
were anesthetized (30 mg/kg sodium pentobarbital), 
and the infrarenal abdominal aorta was exposed. 
The aorta was cannulated, perfused (at a pressure of 80 
mm Hg) with normal saline for 2 minutes, and then 
with fixative (2.5% glutaraldehyde in 0.1 M sodium 
cacodylate buffer) for 10–15 minutes. Both iliac arter-
ies were harvested, and fixation continued for 24 hours 
at 4°C. The artery segments were then critically point 
dried, bisected longitudinally, sputter coated with gold 
and palladium, and examined in a scanning electron 
microscope (AMR 1400).

Immunohistochemical identification of endo-
thelial cells. Staining for factor VIII-related anti-
gen was used to confirm the endothelial origin of the 
lining cells. Vessels were harvested 2 (n = 3) or 4 
weeks (n = 3) after severe injury. Frozen sections 
were fixed with acetone (10 minutes at 22°C) and 
stained for factor VIII-related antigen (goat anti-
human factor VIII–related antigen, Atlantic Antibod-
ies, Scarborough, Maine) with an immunoperoxidase 
technique with rabbit anti-goat immunoglobulin G 
and avidin-biotin complex (Vector Labs, Inc., Burlin-
game, California).

Quantification of intimal proliferation. To provide 
a semiquantitative parameter of intimal proliferation, 
morphometric analysis was performed on vascular 
rings on completion of the organ chamber studies. 
Three epon-embedded cross sections from each ring 
were used to establish a ratio of intimal to medial 
thickness as follows. Each section was photographed 
and then projected onto a digitizing tablet (Zeiss, 
Inc.). Intimal and medial thicknesses were measured 
along 24 polar coordinates for each section with a 
grid superimposed on the projected vascular image. 
The ratio of intimal to medial thickness was then
Figure 1. Light micrographs of rabbit iliac arteries showing morphologic features of endothelial cells. Panel A: Normal endothelial cells (arrowheads) that line the luminal surface of a control vessel. Panel B: Regenerated endothelial cells that are cuboidal (arrowheads) and that line the proliferated intima of an artery 4 weeks after severe injury. Magnification bars represent 40 μm.
FIGURE 2. Light micrographs of rabbit iliac arteries, stained en face with silver nitrate, showing endothelial morphological features. Panel A: Uninjured control vessel. Endothelial cells are aligned in the direction of blood flow (horizontal). Regenerated endothelium 2 weeks (panel B) and 4 weeks (panel C) after severe injury. Regenerated endothelial cells are heterogeneous in size and shape and lack the typical alignment with blood flow. Magnification bars represent 40 μm.
calculated for all measured points, and a mean value for each ring was determined. This ratio is close to zero under normal circumstances because the intima is formed only by a monolayer of flat endothelial cells. Because these vascular rings were not histologically fixed under physiological distending pressure, these measurements are semiquantitative. However, all rings were prepared in the same fashion so that a qualitative assessment of this data is justified.

Organ chamber experiments. After the rabbits were killed with an overdose of pentobarbital, both iliac arteries with the abdominal aorta were removed and immersed in cold Krebs physiological saline solution of the following composition (mM): 118.3 NaCl, 4.7 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂, 25.0 NaHCO₃, 0.05 Ca-EDTA, and 11.1 glucose. After cleaning the vessels of adherent fat and connective tissue, rings of a 5-mm length were cut from the left (experimental) and the right (control) external iliac arteries with iridectomy scissors. Three pairs of rings (experimental and control) from the proximal, central, and distal portions of the iliac artery were studied simultaneously. They were mounted horizontally on stainless steel stirrups in organ chambers filled with 25 ml Krebs solution maintained at 37°C and continuously aerated with 95% O₂-5% CO₂. Isometric tension was measured with a linear force transducer (FT03, Grass Instruments, Quincy, Massachusetts) and recorded on a polygraph (Model 79 D, Grass Instruments). Before study, the rings were progressively stretched for 2 hours until the optimal length-tension relation was achieved as assessed by contraction to norepinephrine (10⁻⁶ M).

Protocol

After the optimal length-tension relation was achieved, vessels were exposed to increasing concentrations of norepinephrine, added to the organ chamber in half-log molar increments. After the maximal response to norepinephrine was obtained, the rings were washed repeatedly with fresh physiological saline solution until tension returned to baseline. To study vasodilation, the vessel was first contracted by a concentration of norepinephrine inducing the half-maximal (EC₅₀) response. Endothelium-dependent relaxations to acetylcholine (10⁻⁸ to 10⁻⁵ M) and to A23187 (10⁻⁸ to 10⁻⁶ M) were studied in the rings from the central portion of the lesion and their corresponding control rings. Relaxations to ADP (10⁻⁷ to 10⁻⁴ M) and nitroprusside (10⁻⁵ to 10⁻⁴ M) and contractions to serotonin (10⁻⁹ to 10⁻⁶ M) and to KCl (20, 40, and 60 mM) were studied in a randomized manner so that the proximal, central, or distal pair of rings was tested with each agent. Because the effects of A23187 on the endothelium may be difficult to reverse, this drug was always the last one tested. Studies of endothelium-dependent relaxation were performed in the presence of indomethacin (10⁻⁵ M) to prevent prostanoid formation.

Drugs

The following drugs were used in the physiological studies: acetylcholine chloride, ADP, calcium iono-
phore (A23187), indomethacin, norepinephrine bitartrate, 5-hydroxytryptamine creatinine sulfate (serotonin), sodium nitroprusside (all from Sigma Chemical, St. Louis, Missouri). All drugs were prepared daily with distilled water except for A23187, which was dissolved in dimethylsulfoxide (DMSO, Sigma). DMSO alone in concentrations up to 0.2% in the organ chamber did not cause any vascular response. The drugs were kept on ice during the experiment.

Analysis of Data and Statistics

Results are expressed as the mean±SEM. Relaxations are expressed as percent changes from the preconstricted level. Contractions were evaluated for the maximal response in grams of tension and for the concentration of drug inducing half-maximal response (EC50). Statistical analysis of contractile response was performed with the Student’s t test for unpaired data. When multiple comparisons were made, an analysis of variance was applied followed by the Scheffe’s test to detect differences among groups. The morphometric data (intimal to medial thickness ratios) were analyzed with a two-way analysis of variance (ANOVA) with severity of injury and time after injury as independent variables in a 2x2 design. The correlation between the intimal to medial thickness ratio and maximal relaxation to acetylcholine was analyzed by linear regression. Statistical significance was set at a p value less than 0.05.

Results

Histological Study

Examination of regenerated endothelium. CROSS SECTIONS. In sections from rings examined on completion of the organ chamber studies, as well as from vessels isolated solely for the purpose of histological study, the luminal lining cells were flat in control vessels and cuboidal in balloon-injured vessels (Figure 1).

SILVER STAINS. Vascular segments stained with silver nitrate were analyzed qualitatively by consensus of two observers. In all animals studied (n=7), regenerated endothelium was present in the balloon-injured areas (Figure 2). In comparison to normal endothelial cells (Figure 2A), regenerated endothelial cells were often irregular in size, polygonal in shape, and lacked the normal longitudinal orientation with blood flow (Figure 2B, C). Small areas of endothelial denudation were seen in specimens of control and balloon-injured vessels and were likely due to artifacts caused by manipulation of the tissue.

SCANNING ELECTRON MICROSCOPY. Scanning electron microscopy of the control (right) iliac arteries revealed an intact endothelial cell lining with fusiform cells aligned in the direction of blood flow and without evidence of intercellular gaps. The balloon-injured left iliac arteries also revealed an intact endothelial cell lining with focal areas of polygonal-shaped and irregular-sized cells not aligned with blood flow. Between these aberrant endothelial cells were gaps in the intercellular borders that often contained adherent intact or metamorphosing platelets (Figure 3).

IMMUNOHISTOCHEMISTRY. In control and balloon-injured iliac artery segments studied at 2 (n=3) or at 4 weeks (n=3) after the severe injury, the luminal cell monolayer stained positively for factor VIII-related antigen (Figure 4).

Analysis of intimal proliferation. The analysis of intimal proliferation 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. In a random sample of six normal iliac arteries, the mean value for the intimal to medial thickness ratio was 0.045±0.005. In balloon-injured arteries, intimal proliferation was present in all vascular rings analyzed (Figure 5A-C, Table 1). Two-way ANOVA revealed a significant effect of severity of injury (F=22.47, p=0.0001), time after injury (F=7.64, p=0.012), and an interactive effect of both variables (F=4.41, p=0.0487) on intimal thickness (Table 1). The internal elastic lamina was disrupted in 19 of 34 rings (56%) after severe injury and only in one of 12 (8%) after moderate injury (Figure 5B and C).

Organ Chamber Experiments

Characterization of rabbit iliac artery. Endothelium-dependent responses of the normal rabbit iliac artery were characterized in preliminary experiments. Acetylcholine, ADP, and A23187 caused concentration-dependent relaxations in rings with intact endothelium, which were abolished after denuding with forceps (n=6 for each drug). In contrast, histamine (10^-4 to 10^-4 M), bradykinin (10^-10 to 10^-5 M), serotonin (10^-9 to 10^-4 M, in the presence and absence of ketanserin 10^-7 M, an 5-HT receptor antagonist), and thrombin (1 and 3 units/ml) did not elicit endothelium-dependent responses.

Acute effects. Vascular rings studied immediately after angioplasty showed negligible contraction to norepinephrine, and relaxation could not be studied. Therefore, vessels were studied 2–3 days after angioplasty (n=3) to confirm loss of endothelium-dependent relaxation in arteries in which endothelial denudation was evident histologically (two severe and one moderate injury). In these rings, maximal contraction to norepinephrine was 35% of control. None of the rings showed relaxation to acetylcholine or A23187 but all relaxed fully to nitroprusside (data not shown).

Effects at 2 and 4 weeks. ACETYLCHOLINE. Concentration-dependent relaxation to acetylcholine was significantly impaired at 2 weeks, after moderate and severe injury (Figure 6). After the severe injury, this attenuation tended to progress between 2 and 4 weeks. In contrast, after moderate injury, relaxations had normalized at 4 weeks (Figure 6).
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FIGURE 3. Scanning electron micrograph of intimal surface from midportion of rabbit iliac artery 3 weeks after angioplasty. The surface is covered by endothelial cells (EC) of irregular size and shape that are not aligned with blood flow. Interendothelial junctions are notable for the presence of interdigitating pseudopodia and the numerous intercellular gaps (large arrows). Scattered cilia-like structures are present on the luminal surface of the endothelial cells (small arrows). A red blood cell (RBC) is present on the luminal surface. Original magnification, ×3,000. Gold and palladium coated.

ADP. At submaximal concentrations, the response to ADP was significantly attenuated at 2 and 4 weeks after severe injury only (Table 2).

A23187. After severe injury, concentration-dependent relaxations to A23187 were significantly reduced compared with relaxations in control rings (Table 2). After moderate injury, at 2 and 4 weeks, relaxations were reduced only at submaximal concentrations.

Nitroprusside. Relaxations of balloon-injured rings to nitroprusside were similar to control relaxations in all four groups (Figure 7).

Norepinephrine, serotonin, and potassium chloride. The EC50 for the contractile agents was not significantly different between control and balloon-injured rings, except for that of norepinephrine assessed at 2 weeks in the moderate injury group (Table 3). Maximal contractions to norepinephrine were reduced at 2 and 4 weeks after severe injury and at 2 weeks after moderate injury; maximal contractions to serotonin were reduced 4 weeks after severe injury. Contractions to potassium chloride were not different from those of controls (Table 3).

Relation Between Morphological Features and Function

For linear regression analysis, all rings that had been subjected to a severe injury (2 and 4 weeks) were included. The intimal thickness correlated inversely with the maximal relaxation to acetylcholine (r = -0.45, p < 0.01).

Discussion

This study demonstrates that a persistent and generalized loss of endothelium-dependent relaxation occurs after balloon injury despite regrowth of endothelium. The degree and the progression of this impairment are critically dependent on the severity of the initial injury. Furthermore, smooth muscle proliferation progresses despite regeneration of the endothelium, and there is a significant correlation between the degree of smooth muscle proliferation and the loss of endothelium-dependent vasodilation. Because nitrovasodilators are now known to inhibit proliferation of vascular smooth muscle in vitro,8 reduced release of EDRF from the aberrant regenerating endothelium may contribute to fibromuscular hyperplasia after balloon injury.
Our observations extend the work of Shimokawa and colleagues, who found functional and histological abnormalities of regenerated intimal cells after balloon denudation of porcine coronary arteries. In their model, endothelium-dependent relaxation was impaired only in response to aggregating platelets and to serotonin, whereas responses to other endothelium-dependent vasodilators were preserved. The present study revealed a broader impairment of endothelium-dependent responses involving receptor- and nonreceptor-mediated agonists. Possible reasons for this disparity include differences in animal species, arterial beds, the severity of balloon injury, and the degree of intimal proliferation. In
either study, the impairment of endothelium-dependent relaxation cannot be explained by persistent injury to the vascular smooth muscle because vasorelaxation to endothelium-independent vasodilators is preserved.

The moderate and severe types of vascular injury induced in this study precipitate migration of smooth muscle cells into the intima, and therefore, a question may arise about the origin of the regenerated cells lining the arterial lumen. Prior studies demonstrated that after extensive vascular injury, a “pseudoendothelium” may be formed by these migrating smooth muscle cells that assume the flat shape of endothelial cells and even provide a nonthrombogenic surface. However, these pseudoendothelial cells do not stain positively for factor VIII–related antigen, a product synthesized specifically by endothelial cells. In our study, the regenerated vascular lining cells were clearly identified as endothelial cells by immunohistochemical staining for factor VIII–related antigen.

The regenerated endothelial cells showed distinct morphological differences from normal endothelial cells, because they were cuboidal rather than flat and lacked the typical orientation in the direction of arterial blood flow. Biological mediators that are known to alter the morphological features of endothelial cells and which are potentially present at sites of vascular injury include cytokines, such as interleukin-1, tumor necrosis factor, and gamma-interferon. These compounds, which are products of mononuclear cells and which can also be produced by endothelial and vascular smooth muscle cells, have been described to transform endothelial cells to an “activated” state, a condition in which endothelial cells develop new properties including increased expression of adhesion molecules for leukocytes and production of procoagulant tissue factor. Furthermore, growth factors known to promote smooth muscle proliferation may also regulate certain aspects of endothelial cell function (transforming growth factor-β and fibroblast growth factor). Maybe this “activation” is also responsible for the impaired endothelium-dependent relaxation observed in this study.

In the present investigation, the degree of impairment in endothelium-dependent relaxation was determined by the severity of, and time elapsed since, the initial injury. Furthermore, it correlated with the extent of intimal proliferation. Regrowth of endothelium was observed at 2 weeks after moderate and severe injury. However, dysfunction persisted only after the latter. Because intimal proliferation was greater after severe than after moderate injury, a partial explanation for this finding is that the thickened intima formed a physical or a functional barrier to the actions of EDRF. This factor has a short half-life that can be reduced further by superoxide anion, a product of macrophages, than may be present at sites of vascular injury. However, because endothelium-dependent relaxation recovered in moderately injured vessels at 4 weeks despite persistence of intimal proliferation, additional factors must be operative.

Alternatively, intimal thickness may simply be a marker of endothelial dysfunction, because factors that lead to endothelial dysfunction (e.g., cytokines and growth factors) may also cause smooth muscle proliferation. The finding of a correlation between intimal thickness and impairment of endothelium-dependent relaxation has been also reported in an atherosclerotic porcine model. In

<table>
<thead>
<tr>
<th>Table 1. Intimal Thickness of Rabbit Iliac Arteries 2 and 4 Weeks After Moderate or Severe Balloon Injury</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>2 wk</td>
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<tr>
<td>4 wk</td>
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<tr>
<td>Severe</td>
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<tr>
<td>2 wk</td>
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<tr>
<td>4 wk</td>
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Six rings were analyzed in each group.

Two-way analysis of variance revealed that the intimal thickness was significantly influenced by the severity of injury and the time elapsed since the injury (see text).
FIGURE 5. Light micrographs of rabbit iliac arteries showing intimal proliferation after injury. Panel A: Control vessel. Panel B: Vessel 4 weeks after moderate balloon injury, showing focal areas of intimal proliferation. Panel C: Vessel 4 weeks after severe balloon injury, showing marked intimal proliferation and disruptions in the internal elastic lamina (arrows). Magnification bars represent 250 μm.
this model, sites of histamine-induced spasm were localized to areas of intimal thickening. The inference from our findings, as well as from previous studies, is that intimal thickening is closely associated with abnormal responsiveness of the arterial wall to a variety of vasoactive agents.

In addition to regulating vasomotor tone, the normal endothelium produces substances that have antiplatelet effects and that inhibit vascular smooth muscle proliferation. Recently, nitrovasodilators were found to inhibit vascular smooth muscle proliferation in vitro. Thus, it may be that the intimal...
TABLE 2. Endothelium-Dependent Relaxations of Rabbit Iliac Arteries After Moderate or Severe Balloon Injury

<table>
<thead>
<tr>
<th></th>
<th>Agonist concentrations (M)</th>
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<tbody>
<tr>
<td></td>
<td>10^-8</td>
</tr>
<tr>
<td>ADP</td>
<td></td>
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<tr>
<td>Control (n=16)</td>
<td>98±1</td>
</tr>
<tr>
<td>2 wk</td>
<td></td>
</tr>
<tr>
<td>Moderate (n=6)</td>
<td>98±1</td>
</tr>
<tr>
<td>Severe (n=6)</td>
<td>100</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
</tr>
<tr>
<td>Severe (n=6)</td>
<td>99±1</td>
</tr>
<tr>
<td>A23187</td>
<td></td>
</tr>
<tr>
<td>Control (n=24)</td>
<td>97±1</td>
</tr>
<tr>
<td>2 wk</td>
<td></td>
</tr>
<tr>
<td>Moderate (n=6)</td>
<td>100</td>
</tr>
<tr>
<td>Severe (n=6)</td>
<td>96±3</td>
</tr>
<tr>
<td>4 wk</td>
<td></td>
</tr>
<tr>
<td>Moderate (n=6)</td>
<td>100</td>
</tr>
<tr>
<td>Severe (n=6)</td>
<td>99±1</td>
</tr>
</tbody>
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Data expressed as the percentage of the initial tension in grams in response to norepinephrine.
*<p<0.05, relaxation in response to ADP or A23187 significantly less than control response.

TABLE 3. Contractile Responses of Rabbit Iliac Arteries After Moderate or Severe Balloon Injury

<table>
<thead>
<tr>
<th></th>
<th>EC50</th>
<th>Maximal contractions (g)</th>
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<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
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<tr>
<td>(log M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.9±0.1 (6)</td>
<td>6.7±0.1 (6)</td>
</tr>
<tr>
<td>Balloon</td>
<td>6.2±0.2 (5)*</td>
<td>6.9±0.2 (6)</td>
</tr>
<tr>
<td>Serotonin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(log M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.3±0.3 (5)</td>
<td>7.1±0.2 (6)</td>
</tr>
<tr>
<td>Balloon</td>
<td>6.6±0.4 (5)</td>
<td>7.0±0.2 (6)</td>
</tr>
<tr>
<td>KCl (log M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>35±5 (4)</td>
<td>...</td>
</tr>
<tr>
<td>Balloon</td>
<td>45±5 (4)</td>
<td>...</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Numbers in parentheses are numbers of rings analyzed.
*<p<0.05, †<p<0.001, ‡<p<0.005, control vs. balloon-injured rings.

proliferation, as well as the reduced endothelium-dependent vasodilation in our model, is due in part to reduced release of the endogenous nitrovasodilator, EDRF. The dysfunctional endothelium regenerating after angioplasty may thus contribute to an imbalance between growth-promoting and growth-inhibiting factors and thus favor restenosis, which is a major problem associated with this procedure.36

In conclusion, the present study shows that after balloon injury of the rabbit iliac artery, the regenerated endothelium demonstrates morphological abnormalities and an impaired endothelium-dependent relaxation to receptor- and nonreceptor-mediated agonists. This impairment is related to the severity of the initial injury and the time elapsed since the injury. The degree of vascular smooth muscle proliferation is related to the severity of the endothelial dysfunction. These observations indicate that the endothelium regenerating after balloon angioplasty may be dysfunctional with respect to its inhibition of vascular tone and growth.

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