Adventitial Angiogenesis Early After Coronary Angioplasty
Correlation With Arterial Remodeling

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Abstract—The spatial correlation between arterial wall microvessels and the accumulation of atherosclerotic plaque is well documented. The role of these microvessels in the development of primary and restenotic lesions is not known. To investigate the effect of interventional procedures on arterial wall microvessels, we studied the adventitial microvascularity of porcine coronary arteries subjected to angioplasty. Twenty-two juvenile domestic swine were subjected to single or repeated (double) balloon angioplasty of the coronary arteries, with the interval between the first and second injury being 14 days. The number, density, and size of adventitial microvessels were measured 1 hour as well as 3, 7, 14, and 28 days after injury. One hour after single balloon injury, there were very few intact adventitial microvessels. Adventitial microvessel number, microvessel area density, and microvessel size were maximal 3 days after single (SI) and double (DI) injury but subsequently underwent progressive regression. Adventitial endothelial cell replication, as assessed by the incorporation of bromodeoxyuridine, was very low for the majority of arteries. Maximal endothelial cell replication indices were observed 3 days after SI and DI (eg, 12.0±3.3%). Early after SI the central arterial lumen area transiently increased, then renarrowed. The lumen area did not change after DI. Arterial remodeling occurred, as the accumulation of intimal and medial mass was correlated with expansion of the external elastic lamina. Adventitial microvessel area density was correlated with central arterial luminal area (R=0.34, P=0.04). The adventitial microvessel area density and the microvessel size index were greater late after DI compared with SI. These data indicate that adventitial angiogenesis occurs within 3 days after balloon injury and that regression of adventitial microvessels after SI corresponds with arterial narrowing. Changes in the adventitial microvasculature may be a component of arterial remodeling after balloon angioplasty. (Arterioscler Thromb Vasc Biol. 1999;19:229-238.)

Key Words: angiogenesis ■ vasa vasorum ■ remodeling ■ restenosis

Angiogenesis, or the “sprouting” of new blood vessels from preexisting ones, requires coordinated proteolysis, endothelial cell (EC) proliferation, and migration. All tissues, including the artery wall, require a microcirculation. Nondiseased arteries have a scant plexus of adventitial vasa vasorum that nourish the inner layers of the artery wall. However, more than a century ago Koester1 described the angiogenesis of a rich network of arterial wall microvessels in atherosclerotic coronary arteries. Later, a number of investigators, including Barger and colleagues,7 have demonstrated that occlusion of adventitial microvessels without additional forms of arterial insult results in neoimtimal formation. These same investigators also promoted the revascularization of adventitial microvessels in devascularized arteries by placing a synthetic tube around the artery and noted a reduction in intimal lesion size.8 Therefore, it is also possible that maintenance of the arterial wall microcirculation is crucial for local tissue homeostasis.

Little is known about the time course of neovascularization and EC proliferation after arterial injury. This information is of potential importance because the abundance of arterial wall microvessels may be critical for the
modulation of arterial repair or for adequate local delivery of therapeutic agents. This study examines the time course and degree of angiogenesis that occur during the response to arterial injury. In addition, we correlate changes in adventitial microvasculature with alterations in the dimensions of the artery wall after injury. Our results indicate that adventitial neovascularization and EC proliferation occur early after balloon injury. The loss of adventitial microvessels after single injury (SI) coincides with the development of arterial narrowing and suggests that changes in the adventitial microvasculature may be a component of arterial remodeling after angioplasty.

**Methods**

A porcine coronary artery model was used to study the change in arterial wall microvessels after balloon injury. All studies were carried out with approval of the University of Ottawa Animal Care Committee and followed the guidelines of the Canadian Council on Animal Care. Twelve-week-old domestic swine were obtained from the University of Ottawa Animal Care Service and fed a standard pig chow (Pig Grower Pellets 9110, Purina Mills). Serial coronary angioplasties were performed via a cutdown incision over the femoral artery. Twenty pigs were equally divided into 4 groups according to the time of sacrifice after the second injury. Two of the 3 major coronary arteries of each animal were subjected to either SI or double injury (DI) with 14 days between the first and second injuries. The remaining coronary artery served as a noninjured control. Animals were humanely killed on days 3, 7, 14, or 28 after SI and DI. In addition, 2 pigs were killed 1 hour after all 3 major coronary arteries were subjected to SI, and the acute adventitial response to balloon injury was studied in these pigs.

**Experimental Procedure**

One hour before surgery, each animal was sedated with ketamine (25 mg/kg) intramuscularly and isoflurane (1% to 3%) by facemask. Atropine (1 mg) was administered intramuscularly to reduce orotracheal secretions. Intravenous access was obtained via an ear vein, and medazolam (1 mg) was given for additional sedation. After endotracheal intubation the animals were ventilated at an initial rate of 15 breaths per minute. Throughout the procedure, anesthesia was maintained with isoflurane (1% to 3%), and the ECG, arterial blood pressure, and arterial blood gases were monitored. A femoral artery cutdown was performed and an arterial sheath inserted. A baseline hematocrit and activated clotting time were obtained. Bretylium (10 mg/kg) was given prophylactically to prevent ventricular arrhythmias. After a 200 U/kg IV bolus of heparin was injected, supplemental heparin was given to achieve an activated clotting time of 300 to 350 seconds. Under fluoroscopic guidance (Cardioscope U/Pan-2000, Siemens) coronary angioplasty was performed in the proximal segment of 1 of the 3 main coronary arteries [ie, left anterior descending (LAD), left circumflex (LCx), and right (RCA) coronary arteries]. The ostium of the left or right coronary artery was cannulated with an angioplasty catheter (8F hockey stick or 8F JR4.0, respectively). After 200 µg of intracoronary nitroglycerin was administered, baseline coronary angiography was performed in 2 orthogonal views by using an iodonic contrast medium (MD 76: 66% diatrizoate meglumine and 10% diatrizoate sodium). A guide wire was advanced into the coronary artery, and an initial injury was performed by using standard balloon angioplasty catheters that were 3.5 to 4.5 mm in diameter. For each artery, the size of the balloon catheter was individually selected to ensure a balloon to artery ratio of 1.3 to 1.5:1. Three balloon inflations at 10 atm for 30 seconds each were performed with a 60-second interval between inflations. After the last angioplasty, 200 µg of nitroglycerin was injected into the coronary arteries and angiograms were repeated. The arterial sheath was then removed, the femoral artery was ligated, and the cutdown incision was closed in 2 layers. Isoflurane anesthesia was then terminated and the pigs were observed in the recovery room before being returned to their stalls. Fourteen days later a second injury was performed on the coronary artery that was originally injured, and another coronary artery was injured for the first time by using the same protocol. Therefore, each pig had 1 SI, 1 DI, and 1 uninjured coronary artery. To label proliferating cells, each pig received a 50 mg/kg IV dose of 5-bromo-2′-deoxyuridine (BrdU; Sigma Chemical Co) dissolved in 0.9% saline 1 hour before euthanasia by KCl overdose.

**Histopathological Processing**

Porcine hearts were harvested immediately after the animals were killed and perfused with Ringers’ lactate at 100 mm Hg for 10 minutes via pressure tubing seated in the ascending aorta. This procedure was followed by perfusion-fixation with 10% neutral buffered formalin at 100 mm Hg for 60 minutes. Hearts were immersion-fixed in 10% neutral buffered formalin overnight and processed the following day. Approximately 30 mm of artery from the proximal LAD, LCx, and mid-RCA angioplasty sites were removed and processed as two 15-mm sections. After fixation, each section was further divided into 3- to 5-mm lengths and embedded in a paraffin block. A minimum of 8 sections from each artery was examined, and the cross section with the most severe luminal narrowing was identified for study purposes. As well, a section of small bowel was resected from each pig and was used as positive control tissue for BrdU immunolabeling.

**Immunocytochemistry**

Immunocytochemical labeling was carried out on adjacent tissue sections by using previously described methods. In brief, this process involved deparaffinizing 5-µm tissue sections, inhibiting endogenous peroxidase activity with 3% H2O2, and applying the primary antibody for 60 minutes at room temperature. Depending on the origin of the primary antibody, a biotinylated anti-rabbit or anti-mouse secondary antibody was applied for 30 minutes, followed by an avidin-biotin-peroxidase complex (ABC Elite kit, Vector Laboratories) for 30 minutes. Tris buffer (0.05 mol/L) containing the standard peroxidase enzyme substrate 3,3′-diaminobenzidine (Sigma) with or without NiCl2 was then added for 10 minutes at 37°C to yield a black or brown reaction product, respectively. Hematoxylin or methyl green was used as a nuclear counterstain. An anti-SM α-actin antibody was used to identify SMCs in the intima and media, as well as myofibroblasts in the adventitia (1:100 titer; Boehringer Mannheim Corp). To identify ECs, tissue slides were predigested with 1.5N HCl for 15 minutes before an anti- von Willebrand factor (vWF) antibody (1:200 dilution; DAKO) was added. As a negative control, PBS containing 1% BSA without the primary antibody was used. Replicating cells were identified with an antibody to BrdU (1:200 dilution; DAKO) was added. As a negative control, PBS containing 1% BSA without the primary antibody was used. Replicating cells were identified with an antibody to BrdU (1:200 dilution; DAKO). Tissue slides were predigested with 1.5N HCl for 15 minutes at 37°C; 0.1 mol/L borax (Sigma), pH 8.5, for 5 minutes at room temperature; and 0.1% trypsin type IX for 7.5 minutes at 37°C. The primary antibody was applied for 2 hours at 37°C. A section of porcine small bowel was used as positive control tissue for the work-up of BrdU immunolabeling. Two independent investigators who were blinded to the nature of tissue sections examined all slides.

**Microvessel Analysis**

A modification of a previous image analysis protocol was used to study neovascularization in these perfusion-fixed porcine coronary arteries. Because the adventitia essentially includes all tissue outside the external elastic lamina, it is therefore difficult to objectively study without applying arbitrary guidelines. In balloon-injured arteries, a neovadventitia (NA) that consists of dense connective tissue and cells consistently forms immediately outside the external elastic lamina. In contrast, the loose connective tissue in the outer adventitia is variable in size and is frequently disrupted during tissue dissection and processing. In uninjured arteries the NA is small. In balloon-injured arteries, the outer border of the NA is easily distinguished from the loose adipose tissue and sparse cellularity found in the outer adventitia. Therefore, the total number of microvessels in the NA, as well as the percentage of NA occupied by microvessels, was objectively studied by 2 observers. Tissue slides of control, SI, and DI arteries from all time points were immunolabeled with an anti-vWF antibody. Seven control arteries and 35
Abundance of Adventitial Microvessels

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<tr>
<th>Sacrifice Interval</th>
<th>Control Arteries</th>
<th>Single Injury*</th>
<th>Double Injury</th>
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<tr>
<td>3–7 + 14–28 Days</td>
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<td>1 Hour</td>
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<td>3 Days</td>
<td>38.00±6.02†</td>
<td>33.00±5.70</td>
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<td>7 Days</td>
<td>38.75±5.22†</td>
<td>34.75±11.32</td>
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<td>14 Days</td>
<td>28.40±4.66</td>
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<td>28 Days</td>
<td>22.60±4.66</td>
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*ANOVA P<0.001. †P value<0.05 compared with control arteries.

balloon-injured arteries were analyzed by using the following protocol. Each artery was divided into quadrants. Under 400× magnification, the optical field within each quadrant that had the most adventitial microvessels was selected for image analysis. Image analysis was performed on coronary artery cross sections by using digitized images projected from an Olympus BX50 microscope via a color camera (Iris CCD, Sony Corp; Bravado Truevision Image-Capturing Software, Jandel Scientific Corp) and analyzed using Mocha image analysis software interfaced with SigmaPlot for Windows (both from Jandel Scientific Corp). To validate this 4-quadrant technique, we compared the results obtained with this method to those obtained using manual counting of all adventitial microvessels. Eight SI arteries were assessed, 2 from each time-interval group (eg, at 3, 7, 14, and 28 days after SI). Using the 4-quadrant method and manual counting techniques, we obtained the following series of data for each of the 4 intervals: 29.5 (15.86±3.94) microvessels. Eight SI arteries were assessed, 2 from each time-interval group (eg, at 3, 7, 14, and 28 days after SI). Using the 4-quadrant method and manual counting techniques, we obtained the following series of data for each of the 4 intervals: 29.5±3.5, 35.0±2.8, 24.0±5.7, and 14.5±0.7 versus 171.0±5.6, 167.5±3.5, 110.5±9.2, and 72.0±21.2, respectively. Therefore, the relative abundance of microvessels calculated using these 2 techniques is similar.

In addition to counting the total number of microvessels per optical field, the perimeter of microvessels within each optical field was manually traced. For each artery the microvascular areas of the 4 quadrants were summed and divided by the total area of the optical fields for all quadrants. This quotient was expressed as a percentage by multiplying by 100, and hereafter is designated the adventitial microvascular area density (MVAD). To determine the average microvessel size for a group of arteries, we divided the total microvessel perimeter by the total microvessel number to obtain the microvessel size index (MVSI). A high index indicates that the average microvessel size is large. The MVSI was expressed in micrometers. Central arterial lumen area (LA), external elastic lamina area (EELA), and the combined area of the intima plus media (I+M) were measured for all cross sections. Because of interruptions of the internal elastic lamina after angioplasty, it was impossible to consider the intima or media as a discrete entity.

EC Proliferation

To quantify the levels of EC proliferation that accompany adventitial microvessel angiogenesis, we used the following strategy. As described in the Results section and the Table, 18 arteries had a moderate to high increase in microvessel number and MVAD relative to uninjured arteries (eg, ≥25% and ≥1.25%; respectively). In a separate study, we counted the total number of cells in the adventitia as well as the total number of adventitial cells that incorporated BrdU and determined that in uninjured arteries, replication is either absent or very low (ie, <0.5%; E.R.O’B. et al, unpublished data, 1998). Therefore, to study EC proliferation in balloon-injured arteries with moderate to high numbers of adventitial microvessels and MVAD, we selected only those arteries with total adventitial cell proliferation indices >0.5%. Twelve coronary arteries met these criteria, 8 of which were harvested 3 days after injury, another 3 arteries were collected 7 days after injury, and 1 artery was harvested 14 days after SI (Figure 1). The entire NA of these 12 specimens was quantitatively analyzed at 400× magnification. The total number of ECs and the total number of BrdU-immunopositive ECs on adjacent cross sections of the same artery were manually counted. The proliferation index (percentage of BrdU-immunopositive ECs) was then calculated for the adventitia of each artery.

Statistical Analysis

Quantitative data were expressed as mean or median±SD. For comparison of multigroup variables, the variance of means was analyzed by 1-way ANOVA. If the F test results were significant, post hoc comparisons were carried out using a Tukey test to perform multiple pairwise comparisons. A Mann-Whitney 2-sample test was used to compare the SI and DI groups. A Pearson correlation test was used to correlate MVAD with LA, EELA, and I+M area. Statistical significance was defined by a value of P<0.05. Changes in arterial wall parameters after SI were compared with control (uninjured) arteries. For DI arteries, arterial wall parameters were compared with SI arteries on day 14.

Results

In the main part of this study, 20 pigs were subjected to the experimental protocol and were equally divided among the 4 sacrifice intervals of 3, 7, 14, and 28 days. Owing to tissue-sectioning artifacts, the adventitial microvasculature could not be analyzed in 3 SI and 2 DI arteries. As well, blood clot obscured the measurement of LA, EELA, and I+M area for 1 additional SI and 4 additional DI arteries. To study the acute effects of balloon injury, 2 additional pigs were subjected to SI of all 3 major coronary arteries 1 hour before sacrifice. All pigs thrived during the study period (ie, baseline body weight on day 0, 23.0±1.3 kg; day 14, 30.8±0.9 kg; day 17, 33.8±3.4 kg; day 21, 35.4±7.6 kg; day 28, 42.8±1.5 kg; and day 42, 52.8±2.9 kg).

Histomorphology

Uninjured coronary arteries were composed of a thin, hypocellular adventitia with sparse vascularity, a modest media, and an intima consisting of rare SMCs covered by a monolayer of ECs (Figure 2A). One hour after SI the intima and media were severely disrupted, usually with a full-thickness dissection of the media in 1 or more locations. The EEL was intact in all arteries at this interval. The adventitia was also traumatized by SI and contained pockets of hemorrhage due to disruption of the vasa vasorum. On days 3 and 7 after SI and DI, we consistently found medial dissections, intramural thrombus, and a marked increase in adventitial dense connective tissue and cells (Figure 2B and 2C).

Adventitial Microvessel Density, Number, and Size Index

Cross sections of 7 control and 35 injured porcine coronary arteries were subjected to 4-quadrant analysis of the MVAD, microvessel number, and MVSI (Figure 3A and 3B). The MVAD after SI and DI is displayed in Figure 4A. The MVAD after SI and DI was significantly different from that of control arteries (P<0.05). Three days after SI, the MVAD increased to 2.89±0.25 (P=0.01 versus control). The MVAD 7 days after SI decreased to 2.14±0.95% and was not different from that of control arteries (P=0.25). On days 14 and 28 after SI, there was further regression of the MVAD to levels similar to those of controls and less than the MVAD of
arteries harvested 3 days after SI (ie day 14, 1.04±0.61%, P=0.008; day 28, 0.77±0.42%, P=0.002).

The MVAD varied after DI (by ANOVA, P=0.001). The MVAD on days 3 and 7 after DI (3.13±0.76% and 2.65±0.63%, respectively) was larger than the MVAD of SI arteries on day 14 (P=0.001 and P=0.006, respectively). Similar to the course described for the SI group, the MVAD had decreased by 14 and 28 days after DI to levels near those of control arteries (1.62±0.74% and 1.68±0.45%, respectively). The MVADs of the SI and DI arteries at the same time points after injury were not different except for 28 days after injury, when the MVAD of the DI group was greater than that of the SI group (P=0.03). The changes in adventitial microvessel number after SI followed a pattern similar to that of the MVAD, increasing early after injury and then decreasing to levels greater than controls by day 14 (the Table). In contrast, the number of adventitial microvessels at all intervals after DI was not different than in SI arteries on day 14.

We considered that the increase in MVAD after injury may have been due to an increase in microvessel number as well as an increase in the size of adventitial microvessels. Therefore, we sought to determine whether changes in vessel size also occurred in response to arterial injury. After injury the MVSI (ratio of MV perimeter to microvessel number; Figure 4B) increased early after SI and DI (by ANOVA, P=0.004 and P=<0.001, respectively). The MVSI on days 3 and 7 after SI (73.2±18.8 and 54.7±13.6 μm, respectively) was greater than the MVSI of control arteries (31.2±10.9 μm, P=0.002 and P=0.012, respectively). Similarly, the MVSI on days 3 and 7 after DI (82.2±12.5 and 76.7±9.3 μm, respectively) was greater than that of the SI arteries on day 14 (43.2±7.3 μm, P<0.001 for both comparisons). Fourteen and 28 days after SI, the MVSI decreased to levels that were similar to those of control arteries. However, the MVSI 14 and 28 days after DI remained elevated compared with the SI arteries on day 14 (58.3±10.7 μm, P=0.031; 66.2±17.8 μm, P=0.032; respectively).

Replication of Adventitial Microvessels
To estimate the degree of adventitial angiogenesis in the minority of specimens that had elevated levels of neovascularization, we used the arbitrary criteria outlined in Figure 1. It is important to note that 28 of the 40 arteries included in this study had low EC proliferation indices and were not included in this detailed analysis. The number of BrdU-immunopositive ECs and the total number of adventitial ECs were manually counted, and the percentage of adventitial ECs that were proliferating was determined. BI indicates balloon injury.

![Figure 1. Analysis of adventitial (Adv.) angiogenesis in porcine coronary arteries. To study adventitial EC proliferation in detail, we used the arbitrary criteria listed on the left to select coronary arteries that had abundant adventitial microvessels and evidence of proliferation. The majority of specimens (28 of 40) did not have a sufficient number of adventitial microvessels or levels of replication to meet our criteria for this detailed analysis of EC proliferation. For the 12 coronary arteries that did meet these criteria, all proliferating adventitial ECs as well as the total number of adventitial ECs were manually counted, and the percentage of adventitial ECs that were proliferating was determined. BI indicates balloon injury.]
of 8 arteries harvested 3 days after SI or DI had elevated levels of EC replication (12.0±3.3%). In this early-injury group the proliferation indices of the SI and DI groups were similar (13.9±1.3% and 10.9±3.7%, respectively). Seven days after SI or DI, only 3 of 7 arteries had elevated EC proliferation indices, including 1 artery with an EC proliferation index of 15.9% in the SI group. Only 1 of 18 arteries from the later time points after injury was analyzed, and this artery was harvested 14 days after SI and had a proliferation index of 10.2%. The majority of the adventitial microvessels and proliferating ECs in this artery were located adjacent to organizing thrombus and a large intimal/medial dissection plane.

**Arterial Areas**

**Central Arterial LA**
The LA varied after SI (by ANOVA, P<0.001; Figure 7A). The LA on day 3 (5.89±1.62 mm²) was greater than that at all other SI time intervals (versus control, 1.90±0.54 mm², P<0.001; versus day 14, 1.71±0.40 mm², P=0.001). The LA at all intervals after DI was similar to that of SI arteries on day 14 (by ANOVA, P=0.12).

**External Elastic Lamina Area**
Changes in the area of the EEL were noted after both SI and DI (by ANOVA, P<0.001 and P=0.014, respectively; Figure 7B). For example, the EELA increased from a control value of 2.59±0.68 mm² to 7.52±1.67 mm² on day 3 after SI (P<0.001). Thereafter, the EELA shrank to 3.28±1.03 mm² by day 7 (P=0.02 versus day 3). The EELA on day 28 after
SI was also larger than control values; however, this finding may reflect normal growth of the artery over time. On days 14 and 28 after DI, the EELA was greater than those in SI arteries on day 14 (\(P < 0.001\) and \(P < 0.002\), respectively).

**T**1 M Area
The T1 M area increased after both SI and DI (by ANOVA, \(P < 0.001\) and \(P = 0.004\), respectively). After SI there was a progressive increase in the T1 M (control, 0.69 ± 0.17 mm² versus day 28, 2.28 ± 0.32 mm², \(P < 0.001\)). After DI the T1 M did not increase until day 14 and remained elevated on day 28 compared with SI day 14 arteries (SI day 14, 1.72 ± 0.23 mm² versus DI day 28, 3.35 ± 0.67 mm², \(P = 0.001\)).

**Relationship Between Arterial Wall Compartments**
Accumulation of T1 M area was correlated positively with expansion of the EELA (\(R = 0.65\), \(P = 0.00001\)). Moreover, changes in the EELA were correlated with LA (\(R = 0.72\), \(P = 0.0000003\)). These data suggest that arterial remodeling with at least partial compensatory enlargement occurred in this model. Finally, the MVAD was also correlated positively with LA (\(R = 0.34\), \(P = 0.04\)) but not with T1 M area (\(R = -0.0016\), \(P = 0.99\)).

**Discussion**
This is the first study to document the kinetics of adventitial angiogenesis after arterial injury. Our results show an early increase in MVAD, microvessel number, and MVSI after both SI and DI. Three days after SI and DI, the average adventitial EC proliferation index was remarkably elevated (12.0 ± 3.3%). In part, arterial remodeling occurred as accumulation of T1 M mass was accompanied by expansion of the EEL. Luminal narrowing occurred early after SI but not after DI. The adventitial MVAD was correlated with the central arterial LA, thereby suggesting that changes in the adventitial microvasculature may be a component of the processes involved in arterial remodeling, particularly after SI.

**EC Proliferation**
The results of the current study indicate that adventitial EC proliferation increases early after balloon injury. EC proliferation was remarkably elevated 3 days after SI and DI. By 7 days after SI or DI, elevated EC proliferation occurred less frequently. Only 1 coronary artery from a later time point after injury (14 days after SI) had a high EC proliferation index. This artery had a major dissection of the intima and media with disruption of the EEL and a large organized...
intramural thrombus. During the angiogenic phase of arterial repair, we observed that both the MVAD and MVSI increased. However, at later intervals both of these parameters regressed. Differences were noted in the regression patterns after SI and DI. For example, the MVAD 28 days after DI was greater than the MVAD observed at the same interval after SI. This observation was paralleled by fact that 14 and 28 days after DI, the size of the microvessels had not shrunk to dimensions similar to those of control (SI day 14) arteries and that DI did not result in a difference in adventitial microvessel number when compared with SI arteries on day 14. The mechanisms responsible for maintaining these microvessels after DI are unknown. Possibly, vasodilatory or angiogenic factors (and their respective receptors) may be upregulated by stimuli associated with injury. From our limited analysis of EC proliferation, we failed to observe differences in replication rates after SI and DI that might explain these differences in MVSI. Alternatively, it is possible that differences in apoptosis after SI and DI may exist, but, this was not specifically addressed in this study. However, Desmouliere et al. showed that ECs in granulation tissue undergo apoptosis as early as 8 days after wounding and reach a maximum apoptosis rate between days 16 and 25 after injury. This time course of EC apoptosis parallels our observation of adventitial microvessel regression that began between days 7 and 14 after balloon injury. Whether the withdrawal of angiogenic growth factors induces apoptosis or stimulation by these factors prevent apoptosis of ECs is unknown.

**Microvessel Regression and Arterial Remodeling**

Little is known about the fate of arterial wall microvessels after balloon injury, and more importantly, whether changes in these microvessels influence the process of luminal narrowing. Amplatz and colleagues examined the short- and long-term effects of balloon injury in canine aortas. Although their studies did not use quantitative methods to assess the abundance of microvessels, they suggested that immediately after injury there was a marked decrease in adventitial microvessels due to rupture, while at later intervals capillary budding was observed. As well, Kwon et al. have reported preliminary data on the arterial microcirculation of balloon-injured pig coronary arteries using microscopic computer tomography. These investigators found an increase in the density of vasa in arteries subjected to balloon angioplasty but did not examine the time course of neovascularization nor the relationship of these microvessel changes to arterial wall dimensions. Therefore, the second aim of this study was to relate the changes in adventitial microvascularity to the problem of luminal narrowing. We found an increase in MVAD, MVSI, and EC proliferation early after SI and DI. The central arterial LA increased early after SI but not after DI. The subsequent loss in LA after SI (but not DI) was paralleled by a decrease in the abundance of adventitial microvessels. It is of interest to note that after DI, the number of adventitial microvessels did not change, nor did the central arterial LA. Moreover, the MVAD and MVSI were greater late after DI compared with SI. Therefore, this observation begs the question: Do arterial wall microvessels directly or indirectly influence central arterial lumen size? Based on the studies by Barker and colleagues, occlusion of adventitial microvessels is associated with neointimal formation, whereas restoration of these same vessels attenuates lesion...
size. However, in the absence of more definitive studies, one can only speculate with regard to possible mechanisms by which adventitial microvessels might influence arterial lumen size. For example, it is possible that the microvascular endothelium is a rich source of vasodilatory factors (e.g., nitric oxide). Alternatively, a rich arterial wall microcirculation may be protective by providing an efficient route for the efflux of cells and noxious factors (e.g., lipoproteins) from the artery wall. Finally, we should consider the possibility that the changes in the arterial wall circulation are simply part of a complex form of wound healing that occurs after injury.14,15

As described by Cohnheim16 in 1889, there are a series of events that are generic to wound healing. An initial event is the invasion of inflammatory cells, fibroblasts, and capillaries into the wound. This results in the formation of granulation tissue. With maturation of the wound, fibroblasts disappear, microvessels regress, and wound contracture occurs.17,18 In this study the changes in the arterial

Figure 7. Arterial areas. Image analysis was used to calculate the central arterial LA, EELA, and I+M area of control as well as SI and DI arteries. Arteries were injured on day 0 (SI). For the DI arteries, a second injury was performed on day 14 after SI. Day 0 for SI arteries represents data from uninjured control arteries. Day 0 for DI arteries represents data from SI arteries on day 14 (i.e., immediately before DI). A, For the LA early after SI, expansion of the LA was followed by a progressive loss in LA over time. *P<0.05 compared with all other SI arteries. The LA did not change after DI (by ANOVA, P=0.12). B, For the EELA on day 3 after SI (but not after DI), the EELA increased and then quickly decreased to supranormal levels for all subsequent intervals. At late intervals after SI and DI, the EELA was greater than that of control arteries. *P<0.05 versus control arteries; $P<0.05$ versus a day-3 SI artery. C, For the I+M area after SI, there was a progressive increase in I+M area, whereas only on days 14 and 28 after DI were the I+M areas greater than those of SI day-14 arteries. *P<0.05 versus controls; $P<0.05$ versus all other SI intervals.
wall microcirculation parallel those observed in a wound. Loss of microvessels may lead to tissue hypoxia and fibrosis. Indeed, disruption of vasa vasorum of nondiseased arteries results in tissue restructuring and loss of arterial compliance. These fibrotic changes may present structural barriers that prevent adequate compensatory dilatation as neointimal mass accumulates. In this study, arterial remodeling with at least partial compensatory enlargement occurred, and the MVAD was correlated with central arterial LA. Therefore, it is intriguing to consider that adventitial microvessel regression may be a mechanism of inadequate arterial remodeling due to insufficient compensatory enlargement (or late contracture). Despite much interest in the occurrence of remodeling in human atherogenesis and restenosis, it is only now that data from this study suggest that preservation of the logical mechanisms that govern this phenomenon. The porcine model was designed to maximize the clinical relevance of arterial injury and repair, differences may be required to prevent excessive arterial scarring. Therefore, understanding the factors involved in EC regression is an important goal.

Finally, there are limitations of this study. Although our porcine model was designed to maximize the clinical relevance of arterial injury and repair, differences may remain. For example, the vascularity of different species is known to vary in health and disease. Second, we have not examined the neovascularization of the intima and media of these arteries and are therefore unable to comment on the potential role of these microvessels in arterial remodeling. However, because plaque neovessels are largely derived from adventitial vasa vasorum, it is likely that changes in plaque neovessels are reflected by adventitial events. Third, although the inverse relationship between microvessel abundance and arterial narrowing is of interest, more definitive studies involving agonists/antagonists of angiogenesis may help clarify the functional role of arterial wall microvessels in remodeling. Recently it has been reported that expression of vascular endothelial growth factor, a known angiogenic factor, is upregulated early after arterial injury in a rabbit model and may be instrumental in inducing arterial wall neovascularization.

In our porcine model, we have preliminary evidence that vascular endothelial growth factor is overexpressed in the adventitia 1 hour after balloon injury (E.R.O’B. et al, unpublished data, 1998).

In summary, this is the first experimental study to provide data on adventitial angiogenesis after coronary artery angioplasty. We have demonstrated an increase in adventitial microvascularity early after balloon injury that is due to formation of new microvessels. At late intervals after SI, regression of adventitial microvessels occurs and parallels the development of central arterial luminal narrowing. In contrast, the number of adventitial microvessels and the central arterial LA do not change after DI. These data suggest that regression of adventitial microvessels may be a component of inadequate remodeling. Moreover, if strategies are to be devised to deliver therapeutic agents to the artery wall via the adventitial microcirculation, then the optimal time window is likely beyond the first day and within the first week after injury. Future studies aimed at preventing microvessel regression may be a novel means of addressing inadequate arterial remodeling after balloon injury.

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References


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