Abstract—Recent studies suggest that arterial remodeling plays an important role in restenosis and that remodeling at the reference site may also occur. To assess the chronic effect of the reference site remodeling on angioplasty results, we evaluated reference site remodeling in an experimental atherosclerotic restenosis model. Histological sections of iliac stenoses and their associated proximal reference segments from 50 atherosclerotic rabbits killed 4 weeks after angioplasty were analyzed. Lumen area (LA), external elastic lamina area (EEL), and intimal plus medial areas (I+M) were measured at the lesion (L) and reference (R) sites. Angiography was performed preangioplasty, immediately postangioplasty, and 4 weeks postangioplasty. Restenosis was defined as an angiographic loss/gain ratio of greater than 50% at follow-up angiography. Twenty-three lesions were restenotic (R+) and 32 were not (R−). There was no difference in reference site diameters (RD) between these two groups at the time of angioplasty. However, RDs were significantly smaller in the R+ group than in the R− group (1.24±0.18 versus 1.52±0.28 mm, n=55, P<.01) at 4-week follow-up. Morphometric analysis also showed a smaller LA(R) in the R+ group (0.85±0.27 versus 1.06±0.37 mm2, n=55, P<.02), whereas there was no difference in I+M(R) between the two groups. EEL(R) significantly correlated with EEL(L), LA(R), and I+M(R) in both groups combined (r=.53, n=55, P<.0001; r=.62, n=55, P<.0001; and r=.86, n=55, P<.0001, respectively). Remodeling can favorably and unfavorably affect both the lesion and the reference sites and appear to occur in parallel and proportionately in both sites. These data suggest that angiographic measurement of late percent stenosis using reference site diameters may lead to an underestimation of the percent luminal narrowing in restenotic lesions because unfavorable remodeling occurs in both the lesion and reference sites in restenotic vessels. (Arterioscler Thromb Vasc Biol. 1998;18:47-51.)

Key Words: angioplasty ■ restenosis ■ arterial remodeling ■ reference site

Restenosis after coronary angioplasty remains one of the major limitations of interventional cardiology. Attempts to modify the restenotic process by pharmacological or mechanical approaches have been largely disappointing and may reflect a basic lack of understanding of the underlying mechanisms involved in the vascular response to injury. Mintz et al1,2 suggested that geometric remodeling of the arterial wall was a more important determinant of late loss after angioplasty than neointimal formation. Support for this hypothesis is found in the accumulating body of studies by other investigators.3–6 The mechanisms of geometric remodeling after angioplasty remain controversial, and in particular, remodeling of the angiographically normal reference site has received little attention. Herman et al7 reported that the untreated reference site, as well as the entire dilated segment, was affected by angioplasty and that narrowing of the reference site diameter was observed among restenotic lesions. The angiographic interpretation of chronic angioplasty results may overestimate or underestimate the percent stenosis if there were significant favorable or unfavorable reference site remodeling. The purpose of the present study was to investigate reference site remodeling and its effect on chronic angioplasty results in an atherosclerotic rabbit model.

Methods

Materials

Ketamine, xylazine, lidocaine, and penicillin were purchased from Butler Co, and sodium pentobarbital from James Brudnick Co.

Atherosclerotic Rabbit Model of Restenosis

Fifty-two male New Zealand White rabbits weighing 3 to 3.5 kg were purchased from Pine Acres Rabbitry (Brattleboro, Vt). The rabbits were subject to the Boston University Institutional Animal Care and Use Committee policies and the NIH Guide for the Care and Use of Laboratory Animals governing animal care and standard euthanasia techniques. The animals were housed in the Boston University Laboratory of Animal Science and quarantined for 7 days before use. Atherosclerosis was developed in both iliac arteries as previously described.8–10 Fifty-two rabbits were subjected to bilateral carotid artery ligation, and 10 rabbits were left undamaged as controls. Eight weeks after ligation, all rabbits were killed with an overdose of sodium pentobarbital through an intravenous catheter. The left iliac arteries were removed for histology, and the right iliac arteries were flushed with saline and filled with formalin.}

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Histological Tissue Preparation

After review of the angiograms to guide sampling, 1-cm segments of both the artery including the midpoint of the lesion and the artery including the reference site ≥10 mm proximal to the dilated region were cut into 10 cross-sectional segments and embedded in paraffin. Five-micron sections were removed from the top of the block and at two additional points 300 and 600 μm deeper into the block than the first section. Thus, each lesion and each proximal reference site was effectively sampled at 30 sites, with an interval of approximately 0.3 to 0.4 mm. Samples were stained with van Gieson's elastin and additional sections were reserved for other staining. The proximal reference sites were selected to be as far from the lesion sites as possible, using the angiogram and superimposed image of the 2.5-mm × 20-mm inflated angioplasty balloon as a guide to ensure that the sampled reference sites were at least 10 mm proximal to the dilated region.

Angiographic Analyses

In the present study, cineangiograms were performed for the following purposes: (1) documentation of the lesion and assessment of angioplasty results; (2) guidance for tissue sampling of the lesion site and the reference site, which was defined as a 1-cm segment of the iliac artery ≥10 mm proximal to the 20-mm dilated region (≥20 mm proximal to the center of the lesion); and (3) validation of equal initial vessel size between restenotic and nonrestenotic subgroups at the time of angioplasty.

Image acquisition was accomplished by using a single-plane Philips Maximus 100 cine system with a 6-inch image intensifier having a 3.8 mm line pair per millimeter resolution (North American Philips). The degree of stenosis preangioplasty, postangioplasty, and at 4 weeks after angioplasty, was measured directly on a Vanguard projector screen (Vanguard Instrument Corp) by two independent, experienced investigators using hand-held digital calipers (Brown and Sharp Manufacturing). The true diameters of the proximal reference site and the lesion site and the percent narrowing were calculated by comparing the minimal lumen diameter (MLD) to a proximal reference segment using a 1-cm reference grid to correct for magnification differences. Restenosis was defined as a loss/gain ratio of ≥50% at the lesion site at follow-up angiography 4 weeks postangioplasty. This definition was used to avoid the effect of change in the diameter of the proximal reference site. Prior studies from our laboratory have shown a highly significant correlation between histological and angiographic lumen area, the latter of which was calculated as $(\pi \cdot \text{MLD}^2)/4$.\(^4\)

Morphometric Analysis

The imaging system consisted of an Olympus microscope (model BH-2) with a solid state CCD video camera (Javelin Electronics) mounted on the eyepiece tube. The video signal underwent eight-bit digitization by a video frame grabber (PCVISION Plus, Imaging Technology) in an IBM-compatible computer, with a resolution of 640 (horizontal) by 480 (vertical) pixels. A 2× objective and a 1× television relay lens were used for all measurements of the images displayed on a high-resolution monitor (Trinitron, Sony), resulting in a pixel size of 45.6 μm².

All sections were examined by two independent investigators blinded to the angiographic results. Digital planimetry of tissue sections was performed using a computer-assisted morphometric program (OPTIMAS, Bioscan Inc.). The lumen cross-sectional area (LA) and the area circumscribed by the external elastic lamina (EEL) were measured directly. Intima plus media (I+M) was calculated by subtracting the LA from the EEL. These measurements and the effects of remodeling on lumen area are diagrammatically represented in a previously published study using this model.\(^3\)

For proximal reference site histology, the mean of the areas of the section with the greatest lumen area from each 1-cm segment cut from the artery over the proximal reference site was used for analysis. This system provides both intraobserver and interobserver variability of <0.5%. Statistical Analysis

All values are expressed as mean±SD. A nonpaired Student’s t test was performed to detect differences between restenotic and nonrestenotic subgroups. The F test was performed for equality of variances. If the F test results were significant, the t test for unequal variances with adjusted degrees of freedom was used. For the univariate analysis, data were entered as continuous variables. Linear regression analysis was performed to assess the relationships between angiographic late loss and the change in reference site diameter from immediately after to 4 weeks after angioplasty, as well as those between EEL, LA, and I+M. A value of P≤.05 was considered statistically significant.

Results

Animal Model

Two rabbits (3 lesions) of 52 rabbits (59 lesions) died before follow-up angiography. One lesion showing acute thrombotic occlusion was excluded from the analysis. Thus, morphometric analysis was performed on 50 rabbits (55 lesions).

Angiography

All vessels showed a successful acute angioplasty result. The average minimal lumen diameter at the lesion sites decreased from 1.05±0.21 mm immediately postangioplasty to 0.65±0.43 mm at 4 weeks follow-up (P<.0001). The average proximal reference site diameter was 1.35±0.21 mm immediately postangioplasty and 1.36±0.26 mm at 4 weeks follow-up. Twenty-three of 55 lesions (41.8%) showed restenosis and 32 (58.2%) did not, with restenosis defined as >50% loss of the initial gain. These results are summarized in Table 1. There was no difference in proximal reference site diameters at the time of angioplasty between the two subgroups (1.36±0.20 mm versus 1.34±0.22 mm in the restenotic and nonrestenotic groups, respectively). However, the mean proximal reference site diameter was significantly smaller in the restenotic subgroup than in the nonrestenotic subgroup at 4 weeks follow-up (1.24±0.18 mm versus 1.52±0.28 mm; n=55; P=.01). There was a significant correlation between late loss at the lesion and the change in proximal reference site diameter described.\(^8\)–\(^11\) Animals were anesthetized by using an intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg), prepared for sterile surgery, and administered 150 000 U penicillin postoperatively for all procedures except the final follow-up angiography. All animals underwent primary iliac artery deendothelialization using a 3F Fogarty balloon catheter and were then placed on an atherogenic diet consisting of standard rabbit chow supplemented with 1.5% cholesterol and 7% peanut oil (ICN, Biomedical). In this model, approximately 50% of the balloon-injured iliac arteries develop significant (>50%) stenoses.

Angiography was performed 6 weeks after initiation of atherogen- esis using a 4F Swan Ganz catheter (Baxter Health Care, Edwards Division) introduced into the right carotid artery. Visualization of the iliac arteries was accomplished by hand injection of meglumine diatrizoate under fluoroscopy. Rabbits with iliac arteries having significant angiographic stenosis (>50% occlusion) underwent balloon angioplasty as previously described,\(^8\)–\(^11\) using a 2.5-mm×20-mm Gruntzig angioplasty balloon catheter (C.R. Bard). The balloon was inflated three times to 5 atm for a 30-second interval during each inflation at the site of maximal stenosis. Thirty minutes after angioplasty, a repeat angiogram was performed to assess whether a successful dilation occurred. Successful angioplasty was defined as ≥20% reduct- ion in percent diameter stenosis with <50% residual stenosis. The animals were allowed to recover and returned to their cages.

Follow-up angiography was performed at 4 weeks postangioplasty as described above. The animals were then euthanitized with an overdose of sodium pentobarbital (120 mg/kg) and the vasculature was perfusion fixed at mean arterial pressure with 10% phosphate-buffered formalin (Fisher Scientific) for histological analyses.

Histological Tissue Preparation

After review of the angiograms to guide sampling, 1-cm segments of both the artery including the midpoint of the lesion and the artery including the reference site ≥10 mm proximal to the dilated region were cut into 10 cross-sectional segments and embedded in paraffin. Five-micron sections were removed from the top of the block and at two additional points 300 and 600 μm deeper into the block than the first section. Thus, each lesion and each proximal reference site was effectively sampled at 30 sites, with an interval of approximately 0.3 to 0.4 mm. Samples were stained with van Gieson’s elastin and additional sections were reserved for other staining. The proximal reference sites were selected to be as far from the lesion sites as possible, using the angiogram and superimposed image of the 2.5-mm×20-mm inflated angioplasty balloon as a guide to ensure that the sampled reference sites were at least 10 mm proximal to the dilated region.
from immediately after to 4 weeks after angioplasty for both subgroups combined ($r=0.56$; *P*<.0001; Fig 1). Thus, the proximal reference site diameter decreased as late loss at the lesion.

**Morphometry**

Table 2 shows the morphometric results of the proximal reference sites in the 55 vessels at 4 weeks follow-up. Lumen area at the proximal reference site was significantly smaller in the restenotic subgroup than in the nonrestenotic subgroup ($0.85 \pm 0.27$ mm$^2$ versus $1.06 \pm 0.37$ mm$^2$; *P*<.02). There was no difference in intima plus media area between these two groups, suggesting that the difference in lumen area was not explained by neointimal formation at the proximal reference site. There was no correlation between proximal reference site luminal area and intima plus media area; however, the proximal reference site external elastic lamina area significantly correlated with luminal area ($r=0.42$; *P*<.0001; Fig 2). This finding emphasizes that late lumen size at the proximal reference site is not determined by neointimal formation alone but is related to the extent of remodeling in this rabbit model. The proximal reference site external elastic lamina area also strongly correlated with intima plus media area ($r=0.86$; *P*<.0001; Fig 3), suggesting that the degree of favorable proximal reference site remodeling may be proportional to neointimal formation at that site. The external elastic lamina areas of the lesion site ($3.65 \pm 0.99$ mm$^2$) and the proximal reference site ($3.39 \pm 1.09$ mm$^2$, mean±SD) significantly correlated for both subgroups combined ($r=0.53$; *P*<.0001; Fig 4) suggesting that remodeling may occur in parallel and proportionately at both the lesion site and the proximal reference site.

**Discussion**

This study provides evidence that the proximal, angiographically normal reference segment remodels both favorably and unfavorably after balloon angioplasty in this atherosclerotic rabbit model. Late lumen size at the proximal reference site was not determined by neointimal plaque formation, defined as histologically determined intima plus media areas, but was related to the external elastic lamina area, suggesting that arterial remodeling occurs in the proximal reference site after angioplasty. The degree of remodeling in the proximal reference site positively correlated with the amount of neointimal plaque formation at that site. The change in lumen size at the proximal reference site from immediately postangioplasty to 4-week follow-up decreased as late loss increased. There was no difference in proximal reference site diameters at the time of angioplasty; however, reference site diameters were smaller in the restenotic group than in the nonrestenotic group at 4 weeks after angioplasty. Furthermore, the proximal reference site diameters were less in the restenotic group and greater in the nonrestenotic group than the mean diameters at the time of angioplasty. Thus, angiographically determined late percent stenosis using proximal reference site diameters can be underestimated and overestimated due to unfavorable and favorable reference site remodeling, respectively.

These results are consistent with several clinical and animal studies. Hermans et al. also reported more prominent narrowing of the reference site in restenotic lesions than in nonrestenotic lesions in a study that analyzed more than 700 patients who underwent angioplasty. These data provide the first clinical evidence that arterial remodeling at the reference site may occur and that narrowing of the reference site may

**TABLE 1. Angiographic Results**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preangioplasty</td>
<td>Postangioplasty</td>
</tr>
<tr>
<td>Restenotic (n=23)</td>
<td>0.52±0.18</td>
</tr>
<tr>
<td>Nonrestenotic (n=32)</td>
<td>0.60±0.24</td>
</tr>
<tr>
<td>P*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are given in millimeters and are mean±SD. Lesion diameter is the minimal lumen diameter at the lesion, reference diameter, the diameter of the proximal normal segment. Restenosis was defined as >50% loss of the initial gain from angioplasty.

**TABLE 2. Results of Morphometric Analyses (Reference Sites)**

<table>
<thead>
<tr>
<th></th>
<th>Lumen Area</th>
<th>I+M</th>
<th>EEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restenotic (n=23)</td>
<td>0.85±0.27</td>
<td>2.34±0.88</td>
<td>3.18±0.99</td>
</tr>
<tr>
<td>Nonrestenotic (n=32)</td>
<td>1.06±0.37</td>
<td>2.52±0.95</td>
<td>3.58±1.09</td>
</tr>
<tr>
<td>P*</td>
<td>&lt;.02</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

I+M indicates intima plus media area at the reference sites and EEL, area circumscribed by the external elastic lamina at the reference sites. Values are given as square millimeters and are mean±SD. Restenosis was defined as >50% loss of the initial gain from angioplasty. Lumen area: histological lumen area at the reference sites.

*Restenotic versus nonrestenotic.

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**Figure 1.** Relation between angiographic loss at the lesion sites and the change in the reference site diameters, defined as the reference site diameter immediately postangioplasty minus the reference site diameter at 4-week follow-up. Solid line represents the regression line for all 55 data, although fewer are shown due to overlap.
contribute to the underestimation of late percent stenosis in restenotic patients. Zamorano et al. observed neointimal thickening of the proximal nondilated reference segment by using intravascular ultrasound in patients undergoing angioplasty. The phenomenon was more frequently reported in patients who developed restenosis. More recently, Kimura et al. reported remodeling of the reference segment within 10 mm of the center of the lesion after angioplasty or atherectomy and found that the process of remodeling in human coronary arteries has some axial length. Post et al. also reported late lumen loss in the reference segment of nonstented arteries 42 days postdilation in an experimental atherosclerotic Yucatan micropig model. Interestingly, stented arteries did not show reference segment late lumen loss in that study.

Neointimal formation in proximal reference segments has been reported by several other investigators. The primary mechanism of neointimal formation and lesion progression was hypothesized to be secondary to the passage of the angioplasty apparatus. In the present study, however, the proximal reference segments were not dilated by angioplasty and a guide wire was not used to position the short angioplasty catheter retrograde across the lesion. Thus, the change in the lumen size at the reference site could not be attributed to plaque formation induced by the angioplasty procedure. In this model, reference site remodeling more likely occurred secondary to changes in hemodynamic factors such as wall stress, shear stress, blood flow, and pressure caused by remodeling and plaque formation at the angioplasty site, although the mechanisms remain to be elucidated. Alternatively, remodeling may exist over a length of the artery occurring maximally at the lesion site and tapering in parallel and proportionately at the proximal reference site. This alternative is supported by the finding that the lesion site and the proximal reference site external elastic lamina areas were strongly correlated and the mean lesion site external elastic lamina area, although not statistically significant, was numerically higher than that of the proximal reference site. In addition, the proximal reference site diameter decreased as late loss at the lesion increased, suggesting parallel remodeling of the lesion and reference sites because the change in luminal diameter has previously been shown to be primarily due to remodeling in this animal model.

A number of possible mechanisms of arterial remodeling have been proposed for de novo atherosclerosis. Increased flow with a concomitant increase in shear stress may lead to adaptive enlargement in nonatherosclerotic arteries, as is found in arteriovenous fistulas. In stenotic arteries, a decrease in flow may result in a decrease in arterial size at the reference site. Other factors, such as changes in collagen metabolism (synthesis, degradation, and reorganization), may also be involved in arterial remodeling at the reference site. Chronic arterial constriction may occur by reorganization of the collagen fibrils during the process of degrading the existing matrix and depositing newly synthesized components.

This study has several limitations. First, its relevance largely depends on the validity of the model to represent the pathophysiology in the human coronary artery after angioplasty. The lesions in this model are typically composed of a mixture of lipid-laden macrophages (foam cells) and smooth muscle cells often with a fibrous cap. Only rarely are features such as calcification and necrosis seen in advanced human atherosclerosis.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Significant correlation between the area circumscribed by the external elastic lamina and the luminal area in the proximal nondilated reference sites. These data suggest that late lumen size at the reference site is not determined by neointimal formation alone but is related to the extent of remodeling in this model that was previously reported for the lesion site.

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Highly significant correlation between the area circumscribed by the external elastic lamina and the intimal plus medial areas in the proximal nondilated reference sites. The data may indicate that favorable reference site remodeling occurs in proportion to the amount of neointimal formation.

![Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** Significant correlation between the area circumscribed by the external elastic lamina in the proximal reference site and in the lesion site. The data suggest that remodeling may occur proportionately over a length of the artery into a nondilated segment.
seen in this rabbit model. On the other hand, the advantage of this model, unlike others, is that angioplasty is performed on hemodynamically significant stenoses containing a large amount of plaque volume. There also is a significant amount of plaque observed at the reference site, as in humans. Second, this histological study cannot assess whether late recoil or arterial enlargement occurs at the reference site because serial measurements were not performed. Third, it is possible that the angioplasty equipment may have been passed through the reference site in some cases, although we attempted to ensure that the reference site was not dilated. Angiographic reference site diameter before angioplasty and immediately after was not different, indicating that the sampling site was not balloon dilated.

We conclude that angioplasty affects both the dilated lesion and the angiographically normal, nondilated proximal reference site. The lumen size at the reference site was significantly smaller in the restenotic group at the time of follow-up angiography. These data suggest that late percent stenosis may be underestimated in restenosis. The difference in the reference site luminal areas between the restenotic and nonrestenotic groups was not explained by plaque formation, suggesting that geometric remodeling at the nondilated proximal reference site may occur.

References
Arterial Remodeling at the Reference Site After Angioplasty in the Atherosclerotic Rabbit Model
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