Differences in Compensatory Vessel Enlargement, Not Intimal Formation, Account for Restenosis After Angioplasty in the Hypercholesterolemic Rabbit Model

Tsunekazu Kakuta, MD; Jesse W. Currier, MD; Christian C. Haudenschild, MD; Thomas J. Ryan, MD; David P. Faxon, MD

**Background** In de novo human atherosclerosis, compensatory vessel enlargement limits the effect of intimal plaque formation on lumen narrowing. We hypothesized that arterial remodeling may also play an important role in determining the chronic lumen size after angioplasty and tested this hypothesis using the hypercholesterolemic rabbit iliac artery angioplasty model.

**Methods and Results** Morphometric analysis of histological cross-sectional areas of vessels from animals killed immediately after angioplasty (acute group, n=11) were compared with the same areas from animals killed 4 weeks after the procedure (chronic group, n=37), when restenosis occurs in this model. The area circumscribed by the internal elastic lamina (IEL) increased by 20% from acute to 4 week follow-up after angioplasty (acute group, 2.36±0.45 mm²; chronic group, 2.84±0.89 mm²). Over the same time period, intimal area increased by 0.82 mm². Despite this increase in intimal area, lumen area decreased by only 0.34 mm² because of the compensatory enlargement of the IEL area. In the chronic group, polynomial regression analysis revealed a quadratic relation between intimal area and lumen area \((R^2=.35, P<.001)\). A lumen area of 0.45 mm² (the nadir of the quadratic relation) was used to divide the chronic group into two subgroups: restenotic (n=21; lumen area, <0.45 mm²) and nonrestenotic (n=16; lumen area, ≥0.45 mm²). By definition, there was a significant difference in lumen area between the two subgroups (0.15±0.15 mm² for restenotic; 0.73±0.18 mm² for nonrestenotic). Surprisingly, the intimal areas in the two subgroups were virtually identical (2.41±0.92 mm² for restenotic, 2.49±0.69 mm² for nonrestenotic, \(P=NS\)). The difference in the lumen area between restenotic and nonrestenotic vessels was a result of the significantly greater IEL area in the nonrestenotic subgroup (3.22±0.83 mm² for nonrestenotic, 2.56±0.84 mm² for restenotic, \(P<.05\)). In both restenotic and nonrestenotic vessels, the IEL area increased with increases in intimal area. In the restenotic arteries, the slope of this correlation was <1, showing inadequate compensatory enlargement for the intimal plaque. In the nonrestenotic vessels, the slope was >1, limiting the effect of intimal plaque on lumen narrowing.

**Conclusions** These data indicate that the iliac artery in an atherosclerotic rabbit model compensates for intimal formation after angioplasty by vessel enlargement. Furthermore, the degree of vessel enlargement is more important than intimal area in determining the chronic lumen size. (Circulation. 1994;89:2809-2815.)

**Key Words** • angioplasty • pathology

Restenosis is one of the major limitations of percutaneous transluminal coronary angioplasty.1-4 Although the mechanisms responsible for this process are not completely understood,5-7 histopathological studies in both animals and humans suggest that migration and proliferation of smooth muscle cells and synthesis of extracellular matrix are central to the healing process of an injured artery and contribute to restenosis.7-12 However, efforts to prevent restenosis by targeting these processes have been largely unsuccessful.4,16-19

Glagov et al20 observed that the left main coronary artery in humans showed an adaptive vessel enlargement in response to progressive plaque expansion. More recently, intravascular and epicardial ultrasound studies have shown that compensatory enlargement occurs throughout the coronary tree and preserves or augments lumen area during the early stages of atherosclerosis.21,22

We hypothesized that compensatory enlargement occurs after angioplasty and that restenosis is not merely a process of intimal formation in response to balloon injury but also a process of vascular remodeling in response to balloon injury and intimal formation. The purpose of the present study was to examine this hypothesis in the atherosclerotic rabbit iliac artery angioplasty model.

**Methods**

Male New Zealand White rabbits weighing 3.0 to 3.5 kg were used for this study, which was approved by the Institu-
tional Animal Care and Use Committee of Boston University Medical Center.

**Induction of Atherosclerosis**

Bilateral iliac atherosclerosis was induced as in previous studies in this model. Animals were anesthetized with ketamine (35 mg/kg IM) and xylazine (5 mg/kg IM). Under sterile technique, the distal femoral arteries were surgically exposed, and an arteriotomy was performed. A 3F Fogarty catheter was inserted 20 cm retrogradely. The balloon was inflated just contact the arterial wall and was then pulled back. This process was repeated three times in each iliac artery. The distal femoral arteries were then ligated, and the wound was closed. All rabbits received penicillin (150 000 U IM) and were placed on a diet consisting of rabbit chow supplemented with 1.5% cholesterol and 7% peanut oil.

**Lesion Documentation and Angioplasty**

Six weeks after atherosclerosis was induced, the rabbits were anesthetized as described above. Supplemental intravenous pentobarbital was used as required. The right carotid artery was surgically exposed, and an arteriotomy was performed. A 4F Swan-Ganz catheter (American Edwards) was advanced under fluoroscopic guidance to a position 1 to 2 cm above the aortic bifurcation. Meglumine diatrizoate was injected by hand at a rate of 1 mL/s for 3 seconds. Cineangiographic images were obtained at a rate of 30 frames per second with a single-plane Philips 6-inch (15.2-cm) image intensifier (North American Philips Corp) with a resolution of 3.8 line pairs per millimeter. A 1-cm grid was placed over the pelvis to allow correction for magnification. Angioplasty was performed if a 50% to 99% iliac stenosis was present, as estimated from a still-frame video image.

For the angioplasty procedure, the mid femoral artery was surgically exposed, and an arteriotomy was performed. A 2.5-mm Gruntzig intraoperative transluminal angioplasty catheter (C.R. Bard) was inserted retrogradely until the balloon was across the site of the greatest iliac artery stenosis. Three 30-second inflations at 5 atm were performed. The result was documented by cineangiography with the Swan-Ganz catheter, with care taken to position the image intensifier at the same height. The catheters were then removed, the arteries were ligated, and penicillin 150 000 U IM was administered. Successful angioplasty was defined as a >20% decrease in percent diameter stenosis. Immediately after angioplasty, 11 rabbits (the acute group, 11 vessels) were killed with an overdose of pentobarbital. The iliac arteries were perfused with 10% buffered formalin at 80 mm Hg for 15 minutes via a cannula placed in the aorta. The aorta and iliac arteries were then removed and placed in 10% buffered formalin. Thirty-four rabbits (the chronic group, 40 dilated vessels) were allowed to recover and were maintained on the 1.5% cholesterol diet for 4 weeks, when a final angiogram was performed as described above. After the rabbits were killed, the arteries were perfusion fixed in the same manner as the acute group, then removed and placed in 10% buffered formalin.

**Tissue Preparation**

After review of the angiograms to guide sampling, a 1-cm segment of the artery including the lesions was cut into 10 cross-sectional segments and embedded in paraffin. Five-micrometer sections were removed from the top of the block and at two additional points 300 and 600 μm deeper into the block from the first section. Samples were stained with Van Gieson elastic, and additional sections were reserved for other staining. Since sections from three locations within each block were processed, each lesion was effectively sampled at 30 sites with an interval <0.4 mm.

**Angiographic Analysis**

Cineangiograms were reviewed on a Vanguard projector (Vanguard Instrument Corp) independently by two experienced investigators. Reference and luminal diameters were measured with hand-held digital calipers (Brown and Sharpe Manufacturing) to determine the most stenotic portion of the iliac artery before and immediately after the angioplasty procedure in both the acute group and the chronic group and additionally at 4-week follow up in the chronic group. True diameters were calculated by correcting for magnification with the use of a 1-cm grid.

To assess the relation between angiographic and histological lumen areas in the chronic group, angiographic lumen area was calculated by the formula $\pi(MLD/2)^2$, where MLD is minimal angiographic lumen diameter.

**Morphometric Analysis**

The imaging system consisted of a Olympus microscope (model BH-2) with a solid-state CCD videocamera (Javelin Electronics) mounted on the eyepiece tube. The video signal underwent eight-bit digitization by a video frame grabber (PCVISION Plus, Imaging Technology) in a IBM-compatible computer with a resolution of 640 (horizontal) by 480 (vertical) pixels. A $\times 2$ objective and a $\times 1$ 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 $6.5\,\mu m^2$.

All sections were examined by two investigators blinded to the angiographic results. Digital planimetry of tissue sections was performed with a computer-assisted morphometric program (OPTIMAS, Bioscan Inc). The areas within the lumen and the areas circumscribed by the internal elastic lamina (IEL area) and the external elastic lamina (EEL area) were measured directly. The areas of the media and intima were calculated by subtraction: medial area equals EEL area minus IEL area; intimal area equals IEL area minus lumen area. In all lesions, the single 1 of 30 sections that demonstrated the smallest lumen area was selected for further analysis. This system provides both intraobserver and interobserver variability of <0.5% (unpublished data).

**Statistical Analysis**

All values are expressed as mean±SD. A paired $t$ test was used to detect differences between angiographic minimal lumen diameter immediately after angioplasty and at 4-week follow-up in the chronic group. The statistical significance of differences between acute and chronic groups and between the two subgroups in the chronic group was determined with a nonpaired Student's $t$ test. 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. Linear regression analysis was used to evaluate the relation between angiographic lumen area and histological lumen area as well as the relation between variables in the chronic group. Polynorninal regression analysis was performed to assess the relation between intimal area and lumen area in the chronic group. A value of $P<.05$ was considered statistically significant.

The sample size of the chronic group was determined to give a power of 0.80 to detect a 30% difference in intimal area between restenotic and nonrestenotic subgroups. This calculation assumes a 50% restenosis rate and an intimal area of 2.4±0.7 mm$^2$, as seen in prior studies using this model. The sample size of the acute group was chosen to give a power of 0.80 to detect a 0.2-mm difference between lumen diameters before and after angioplasty compared with a chronic group consisting of the required sample size as determined above. Assumptions in this calculation include equal variances and lumen diameter SD of 0.2 mm, as expected from prior studies using this model.
TABLE 1.  Angiographic Results Before and After Angioplasty

<table>
<thead>
<tr>
<th></th>
<th>Preangioplasty Diameter, mm</th>
<th>Postangioplasty Diameter, mm</th>
<th>Follow-up Diameter, mm</th>
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<tbody>
<tr>
<td></td>
<td>MLD Reference</td>
<td>MLD Reference</td>
<td>MLD Reference</td>
</tr>
<tr>
<td>Acute group (n=11)</td>
<td>0.61±0.22 1.43±0.21</td>
<td>1.12±0.21 1.44±0.25</td>
<td>0.65±0.44 1.39±0.27</td>
</tr>
<tr>
<td>Chronic group (n=37)</td>
<td>0.62±0.23 1.45±0.19</td>
<td>1.10±0.23 1.42±0.23</td>
<td>NS NS</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

MLD indicates minimal lumen diameter; Reference, diameter of normal segment. P values are for acute group vs chronic group. All data are expressed as mean±SD.

Results

Nineteen rabbits had no significant lesions at the time of initial angiography and did not undergo angioplasty. Unilateral 50% to 99% iliac stenoses were present in 39 rabbits, and bilateral 50% to 99% stenoses were present in 6 rabbits. Angioplasty was successful in all 51 of these lesions. Two rabbits (each with one lesion dilated) in the chronic group died during the follow-up period. One lesion in the chronic group, which showed acute thrombotic occlusion, was excluded from the analysis. Thus, morphometric analysis was performed on 94% of vessels undergoing angioplasty, including 11 rabbits (11 vessels) in the acute group and 31 rabbits (37 vessels) in the chronic group.

Angiography

Quantitative angiography was performed to ensure that the acute and chronic groups were comparable in minimal lumen diameter and reference lumen diameter at the time of angioplasty as well as in the immediate angiographic result of angioplasty. As shown in Table 1, there was no significant difference in minimal lumen diameter before or immediately after angioplasty between the two groups (from 0.61±0.22 to 1.12±0.21 mm for the acute group and from 0.62±0.23 to 1.10±0.23 mm for the chronic group). The reference vessel diameter was also similar for both groups (1.43±0.21 mm for the acute group and 1.45±0.19 mm for the chronic group). At 4-week follow-up study, the average minimal lumen diameter for the chronic group animals decreased to 0.65±0.44 mm (P<.0001 versus immediately after angioplasty). The mean stenosis at follow-up angiography showed a significant increase compared with immediately after angioplasty (56.4±26.5% versus 22.7±9.3%), similar to prior angiographic studies in this model.26

Fig 1 shows the highly significant correlation between angiographically calculated and histologically measured minimal lumen area (r=.85, P<.0001). The β-coefficient of 0.64 is probably a result of shrinkage of vessels during histological processing. Since the acute and chronic groups were angiographically similar at the time of angioplasty and angiographic lumen area was highly correlated with histological lumen area, comparison of the histological areas in the acute and chronic groups was then performed.

Morphometry

Table 2 shows the morphometric results of the acute and chronic groups. The area circumscribed by the IEL was 20% (0.48 mm²) larger at 4-week follow-up after angioplasty. This increase accommodated 58.5% of the increase in intimal area (0.82 mm²) over the same time period. As a result, lumen area decreased only 0.34 mm². The EEL area also increased by 20%, and there was a smaller but significant increase in medial area (0.15 mm²).

Fig 2 shows the relation between the intimal area and lumen area for all chronic group vessels; a biphasic relation was seen. At smaller lumen areas, as lumen area decreased, intimal area increased. In contrast, at larger lumen areas, intimal area increased with increasing lumen area. There was no significant linear correlation between the two variables, but polynomial regression analysis confirmed a quadratic relation between intimal area and lumen area (Fig 2, r=.59, P<.001).

To further assess the relation between lumen area and intimal area, we divided the chronic group into two subgroups based on the lumen area at the minimum point of the quadratic equation generated by the polynomial regression model (Fig 2). The two subgroups were designated restenotic (lumen area <0.45 mm², n=21) and nonrestenotic (lumen area >0.45 mm², n=16); they are compared in Table 3. Reference artery angiographic diameter at the time of angioplasty was the same in both subgroups (1.39±0.16 mm for restenotic, 1.42±0.21 mm for nonrestenotic). There was no difference in mean intimal area between the two subgroups, whereas IEL area and EEL area were both significantly greater in the nonrestenotic subgroup. Similar results were obtained by use of mean or median histological lumen area of the entire chronic group to divide the population into restenotic and nonrestenotic subgroups or by use of conventional dichotomous angiographic definitions of restenosis (such as loss of one
a half of the angioplasty gain or 50% angiographic restenosis at follow-up).

Fig 3 shows the relation between IEL area and intimal area in these two subgroups. For both restenotic and nonrestenotic vessels, IEL area correlated strongly with intimal area (r=.99, P<.0001). In the restenotic subgroup, IEL area increased 0.91 mm² (SEE=0.12) for every 1-mm² increase in intimal area. In the nonrestenotic subgroup, IEL area increased 1.22 mm² (SEE=0.13) for every 1-mm² increase in intimal area. The β-coefficients of these two regression analyses were significantly different (95% confidence intervals, 0.84 to 0.98 and 1.11 to 1.30, respectively). This indicates that in nonrestenotic vessels, compensatory enlargement allowed for increased lumen area despite increasing intimal area. Conversely, in restenotic vessels, a smaller increase in IEL area for any given increase in intimal area resulted in a more significant reduction in lumen area. Similar results were obtained by analyzing the relation between EEL area and intimal plus medial area.

Qualitatively, the histological appearances of the lesions in the two subgroups were similar and not different from previous descriptions of restenosis in this model.25,26 There was no apparent clinical difference between the two subgroups and no difference in body weight at follow-up.

TABLE 2. Morphometric Analysis of Most Severely Stenotic Sections

<table>
<thead>
<tr>
<th></th>
<th>Acute Group (n=11)</th>
<th>Chronic Group (n=37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area, mm²</td>
<td>0.74±0.19</td>
<td>0.40±0.34</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Intimal area, mm²</td>
<td>1.62±0.54</td>
<td>2.44±0.82</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Medial area, mm²</td>
<td>0.72±0.15</td>
<td>0.87±0.32</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>IEL area, mm²</td>
<td>2.36±0.45</td>
<td>2.84±0.89</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>EEL area, mm²</td>
<td>3.08±0.47</td>
<td>3.71±0.98</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

IEL area indicates area circumscribed by internal elastic lamina; EEL area, area circumscribed by external elastic lamina. P values are for acute group vs chronic group. All data are expressed as mean±SD.

Discussion

This study provides evidence that compensatory enlargement of rabbit atherosclerotic iliac arteries occurs after angioplasty. The magnitude of the effect of vascular remodeling on late outcome after angioplasty is shown in Fig 4. For simplicity, only the intimal area and IEL area are considered; similar results are obtained with intimal plus medial area and EEL area. In this model, the IEL area increased by 20% during 4-week follow-up, such that lumen area decreased by only 0.34 mm² (46%) despite a 0.82-mm² increase in intimal area (Fig 4A). If the IEL area had increased by only 10% instead of the observed 20%, with the same increase in intimal area, lumen area would have been reduced by 0.58 mm², or 78% (Fig 4B). If no compensatory enlargement occurred, with the same amount of intimal formation, all of the arteries would have been totally occluded.
to ours that, compared with the reference site, there was only a 33% increase in intimal plus medial area but a 52% reduction in lumen area. The comparison of lesion site with reference site is complex, since it is known that the reference site will also show compensatory enlargement over time in response to the initial injury and high-cholesterol diet.30 In the present study, reference segment intimal area at 4-week follow-up study was greater than in the acute reference segment, but a similar nonsignificant increase in IEL area led to near preservation of both histological and angiographic reference lumen area at follow-up.

Two possible mechanisms for vascular remodeling in de novo atherosclerosis have been postulated by Glagov and others. Nonatherosclerotic arteries exposed to increased flow show adaptive enlargement, probably due to an increase in wall shear stress.31,32 Similarly, where atherosclerotic plaques form, compensatory enlargement could be initiated by the increased shear stress that results from the initial stenosis.33-35 Most atherosclerotic plaques tend to be eccentric, and the relatively uninvolved segment of artery wall is likely to respond to the increased shear stress, resulting in arterial enlargement.11,32,36 Alternatively, as plaque develops, it may cause involution of the support structure of the arterial wall.20 Pathological studies37-38 showing outward bulging of the plaque and underlying arterial wall in arteries with eccentric lesions support this mechanism.

In this model, lesions are generally concentric. However, after balloon injury, as in human coronary arteries, disruption of the intima and media are common, and disruption of the adventitia can occur.24 This vascular injury may allow enlargement of a segment of the arterial wall. In addition, the cellular response associated with the healing response to balloon injury may result in the release of proteolytic enzymes from inflammatory cells and initiate the remodeling process. Thus, either of the potential mechanisms described for remodeling in de novo atherosclerosis may be operative after balloon injury.

There is increasing evidence that vascular remodeling occurs after angioplasty in humans. A preliminary intracoronary ultrasound study of 20 patients by Kovach et al39 suggested that intimal formation accounted for only 32% of late lumen loss and that “chronic recoil” with a mean 7.5% decrease in total vessel area (corresponding to EEL area) was more important. These findings were confirmed by the same authors in a larger series of patients in whom arterial remodeling accounted for 60% of late lumen loss.40 Our data suggest that since some degree of intimal formation occurs in response to balloon injury, failure of the artery to enlarge adequately after angioplasty is sufficient to produce restenosis. The phenomena of “late recoil” and “geometric remodeling” described by Kovach39 and Mintz40 may be only one end of the spectrum of vascular remodeling after angioplasty. Our study cannot address whether late recoil occurred in individual arteries, since serial measurements in the same artery were not performed. Even in the restenotic subgroup, average IEL area was greater than in the acute vessels.

The importance of vascular remodeling in human restenosis remains to be determined. A recent study by O’Brien et al41 showed that actual proliferation in restenotic coronary artery lesions occurs at low levels,
raising questions as to the role of intimal hyperplasia in restenosis.\textsuperscript{6} Intimal formation certainly occurs in restenosing coronary arteries after angioplasty,\textsuperscript{3,11,14} but it may also be seen in arteries that do not restenose.\textsuperscript{12} Our data suggest that other pathophysiological mechanisms such as vascular remodeling may be playing a significant role.

The present study has several limitations. First, this model uses i liac, not coronary, arteries, and the histology of the atherosclerotic and restenotic lesions has several differences from that in humans.\textsuperscript{24,42,43} On the other hand, the advantage of this model is that, unlike other restenosis models, angioplasty is performed on hemodynamically significant stenoses containing a large amount of plaque volume. The presence of intimal and medial disruption in a severely diseased artery after angioplasty in this model may better mimic the flow and shear-stress conditions seen after coronary angioplasty in humans. This may be an important factor in assessment of arterial remodeling. Second, the quantitative morphometric analysis used different animals for the acute and the chronic groups. Angiographically, however, the acute and chronic groups were similar before and immediately after angioplasty, and angiographic and histological lumen areas correlated closely. Future studies using intravascular ultrasound may be able to assess arterial remodeling of the same artery over time. Third, despite the correlation between histologically and angiographically determined minimal luminal area, the most severely narrowed histological sections used for the analyses in the present study may not exactly represent the most narrowed segment documented by angiography because of sampling error.

We conclude that arterial enlargement occurred after angioplasty in the atherosclerotic rabbit model and that differences in vascular remodeling initiated by balloon injury played a more important role than differences in intimal formation in determining chronic lumen size. Restenosis after angioplasty may reflect the failure of adequate compensatory enlargement for the unavoidable response to balloon injury, consisting of platelet deposition, thrombus formation, and intimal formation. If we are not able to inhibit these responses to balloon injury, therapeutic strategies to modulate arterial remodeling may be required to reduce restenosis after coronary interventions. Preliminary data using vitamins C and E in the swine balloon injury model suggest that altering vascular remodeling is a possible therapeutic target.\textsuperscript{44}

\section*{Acknowledgments}
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