Influence of Inflation Pressure and Balloon Size on the Development of Intimal Hyperplasia After Balloon Angioplasty

A Study in the Atherosclerotic Rabbit

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To evaluate the effect of balloon size and inflation pressure on acute and subsequent outcome following balloon angioplasty (BA), 70 New Zealand White rabbits with bilateral femoral atherosclerosis were assigned to four groups: group 1, oversized balloon, low inflation pressure (n=35 vessels; balloon size, 3.0 mm/inflation pressure, 5 atm); group 2, oversized balloon, high inflation pressure (n = 36; 3.0 mm/10 atm); group 3, appropriate size, low inflation pressure (n=17; 2.5 mm/5 atm); and group 4, appropriate size balloon, high inflation pressure (n=19; 2.5 mm/10 atm). Angiograms were obtained before, 10 minutes after, and 28 days after BA and read by two blinded observers using electronic calipers. The in vivo balloon-to-vessel ratio was measured for each group. There were eight non-BA controls. Rabbits were sacrificed either immediately (n=34) or at 28 days after BA (n=36), with the femoral vessels pressure perfused for histologic and morphometric analysis. The latter was performed at 28 days only. Absolute angiographic diameters increased in all groups immediately after BA (p <0.01). Acute angiographic success, defined as greater than 20% increase in luminal diameter, was higher using high inflation pressure (group 2, 32/36 [89%] and group 4, 16/19 [84%] vs. group 1, 23/35 [66%] and group 3, 9/17 [53%]; p<0.05). A 3.0-mm balloon resulted in significant oversizing irrespective of inflation pressure (balloon-to-vessel ratio, 1.5±0.1 vs. 1.1±0.1 to 1, for the 2.5-mm balloon). Vessels exposed to high inflation pressure had a significantly higher incidence of mural thrombus, dissection (p<0.01), and medial necrosis versus low pressure (p<0.05). At 28 days, the rates of restenosis (defined as greater than 50% loss of initial gain) were 14/20 (70%), 11/16 (69%), 5/10 (50%), and 5/10 (50%) for groups 1 through 4 (p=NS; a trend in favor of the groups using an oversized balloon). There was an increase in the degree of intimal hyperplasia by morphometric analysis in all groups, being most marked in group 2 (oversized balloon and high inflation pressure, 1.7±0.9 vs. 0.5±0.2 mm for controls, p<0.001). We reached two conclusions. First, all protocols resulted in a significant increase in luminal diameter immediately after angioplasty with the highest success rate in vessels subjected to high pressure dilatation. Second, the combination of high inflation pressure and an oversized balloon caused the most extensive vessel-wall damage early after BA and the greatest degree of intimal hyperplasia at 28 days. Thus, oversizing the balloon with high inflation pressure may represent a factor causing restenosis following BA. (Circulation 1989;80:1029-1040)
Percutaneous transluminal angioplasty is an alternative to surgical revascularization in patients with atherosclerotic vascular disease. Although technical advances have favorably influenced the acute angiographic success and complication rate following angioplasty, a similar impact on the incidence of restenosis has not been observed. The noteworthy exception is the addition of n-3 fatty acids to the diets of patients undergoing coronary angioplasty. Factors responsible for the high restenosis rate remain undetermined. A number of suggested mechanisms based on procedure-related variables have been proposed but most have never been rigorously evaluated. Procedure-related factors thought to impact on restenosis include the balloon size and maximal inflation pressure.

Influence of Balloon Size on Outcome

The optimal balloon size for angioplasty has not been defined. Sizing represents a complex interaction of the specified diameter, balloon composition, inflation pressure, and resistance offered by the diseased vessel wall. Published studies address both acute outcome and restenosis and reveal considerable controversy. Duprat et al concluded that the optimal early angioplasty result was achieved with a balloon-to-vessel ratio of 1.1–1.3 to 1, whereas Schmitz et al reported that balloon size did not influence early angiographic success. With respect to late outcome, there is again no consensus, with Schmitz et al reporting a lower restenosis rate using a larger balloon and Hoffmeister et al finding that restenosis was not influenced by balloon size. A prospective randomized study at Emory University Hospital, comparing an appropriately sized balloon to the next largest size, was terminated because of an unacceptably high incidence of emergency bypass surgery (twice as frequent) and acute myocaridal infarction in patients randomized to larger balloons. The restenosis rates at 6 months in the two groups were similar.

Influence of Inflation Pressure on Outcome

The range of inflation pressures used in current practice is empirical and usually incremental to achieve “loss of waist” in the balloon and a reduction in the translesion pressure gradient. Data from the percutaneous transluminal coronary angioplasty (PTCA) registry of the National Heart, Lung, and Blood Institute and a retrospective study by Meier et al both suggested that higher balloon-inflation pressure during angioplasty was associated with an improved immediate hemodynamic result and speculated that higher inflation pressure may be a factor in improving long-term results. The report by Levine et al demonstrating a higher restenosis rate with inflation pressures less than 8 atm supports that hypothesis. However, an increased restenosis rate has been reported with high inflation pressure in two retrospective studies.

Further complicating the picture are the studies of DiSciascio et al and Hoffmeister et al who found that restenosis was not influenced by inflation pressure. Thus, the optimal inflation pressure that will achieve both the best early success and lowest restenosis rate is unknown.

Proposed Study

Because the questions of optimal balloon size and inflation pressure for angioplasty remain controversial, we studied these variables by inducing bilateral focal femoral atherosclerosis in 70 New Zealand White rabbits and assigned them to transluminal balloon angioplasty with either a 3.0- or 2.5-mm balloon. Each femoral artery was dilated with either 5 or 10 atm inflation pressure. These values were chosen to compare appropriate versus oversized balloons and high versus low inflation pressure for values that cover the range used clinically. Thirty-four animals were sacrificed acutely after angioplasty, and 36 were maintained for 28 days to assess long-term (angiographic and histologic) results.

Methods

The study design is summarized in Figure 1.

Induction of Focal Atherosclerosis

Bilateral focal femoral atherosclerosis was induced by endothelial damage using desiccated nitrogen
gas followed by a 28-day high cholesterol diet.\textsuperscript{31,32} Seventy male New Zealand White rabbits weighing 8–10 lb were anesthetized with ketamine 35 mg/kg (Ketaset, ketamine HCl veterinary injection, Veterinary Products, Bristol Lab) and xylazine 5 mg/kg (Rompun, Bayvet Division, Miles Laboratories Inc, West Haven, Connecticut), both administered by intramuscular injection. Using sterile technique and gentamicin antibiotic cover (dose, 1 mg/kg by intramuscular injection, gentocin (Ketaset, Schering Veterinary, Maplewood, New Jersey), segments of both femoral arteries approximately 1 cm below the inguinal ligament and between 1–2 cm in length were isolated between airtight ligatures. Local spasm was prevented by the topical administration of lidocaine 10 mg (Anthocaine injection 2%, Anthony Products Company, Arcadia, California). The isolated femoral artery segments were cannulated with a 27-g needle and a posterior vent created by needle puncture. The arterial segments were flushed with saline and endothelial damage was induced by the passage of industrial nitrogen gas infused at a rate of 80 ml/min for 8 minutes. Following air-drying, the ligatures were removed and hemostasis achieved by local pressure. The injured segments were demarcated with metal clips (25 hemoclips, medium, catalog no. 523100, Edward Weck & Co., Inc., Research Triangle Park, North Carolina) applied to the adjacent muscle, and the wound was closed with a 4.0 vicryl subcuticular suture. The following surgery, the animals were placed on a 2%-cholesterol and 6%-peanut-oil (Dyets, Inc., Bethlehem, Pennsylvania) diet for 1 month.\textsuperscript{32,33}

From the day of angioplasty, the diet consisted of standard rabbit chow (Dyets, Inc.). Angioplasty was performed 24±3 days after ending the diet. By analysis of variance there were no intergroup differences. Total cholesterol was measured at the time of ischemic injury and at the time of angioplasty.

The rabbits were housed according to the Animal Welfare Act specifications, and all surgical procedures were performed using general anesthesia and standard sterile techniques.

\textbf{Angioplasty}

Rabbits were anesthetized using ketamine (35 mg/kg) and xylazine (5 mg/kg) given intramuscularly, and maintenance anesthesia was achieved with intravenous ketamine (8 mg/kg) and xylazine (1 mg/kg) administered via a marginal ear vein. The first 46 rabbits underwent balloon angioplasty using an oversized 3.0-mm balloon, and the next 24 were dilated with an appropriately sized 2.5-mm balloon. For each balloon size, arteries were randomized to either 5 or 10 atm inflation pressure, and we were, thus, able to avoid a catheter change and prevent unnecessary trauma to the vessel (group 1: 3.0-mm oversized balloon, 5-atm low inflation pressure \(n=35\); group 2: 3.0-mm oversized balloon, 10-atm high inflation pressure \(n=36\); group 3: 2.5-mm matched balloon, 5-atm low inflation pressure \(n=17\); group 4: 2.5-mm matched balloon, 10-atm high inflation pressure \(n=19\)). Thirty-three vessels were totally occluded prior to angioplasty and were excluded from the study. Through a midline neck incision, the right common carotid artery was isolated by blunt dissection and the distal end ligated. Via an arteriotomy, a 4F preformed introducer was inserted and advanced to the junction of the aortic arch. A 0.014-inch USCI Veriflex guidewire (C.R. Bard, Inc., Billenica, Massachusetts) was then introduced and advanced into the descending aorta. A Medi-Tech 3.0 or 2.5–2/4.0/120 (2.0-cm balloon length, 4F catheter size, 120-cm catheter length) polyethylene balloon angioplasty catheter was introduced and positioned approximately 3 cm above the aortic bifurcation. After blood was drawn for baseline activated partial prothrombin time (PTT), 100 units/kg heparin (heparin sodium injection, USP, Elkin-Sinn Inc.) and 20 mg lidocaine were injected intra-arterially. With the rabbit supine and the hind-legs abducted and externally rotated at the hips and the knees extended (this position was maintained using a specially constructed perspex brace), 3–4 ml of Renografin 76 (diatrizoate meglumine and diatrizoate sodium injection USP, Squibb) were injected by hand over 3 seconds to obtain a control angiogram of the distal aorta, iliac, and femoral arteries using a Siemens Tridoros 5.3-phase, 6-pulse radiograph generator and a Siemens biangulux radiograph tube with a 0.6 focal spot. The Kodak X-Omat K film was developed using a Kodak RP X-Omat Processor (model M6-N). The angioplasty catheter was then advanced over a steerable, soft tipped guidewire, and the correct balloon placement across the stenosis was verified using fluoroscopy and the previously placed metal clips as markers.

Three 1-minute balloon inflations, 1 minute apart at 5 or 10 atm, were performed under fluoroscopic vision using a hand inflator (ACS Indeflator, Advanced Cardiovascular Systems Inc., Mountain View, California). Following balloon dilatation, the angioplasty catheter was withdrawn into the abdominal aorta and positioned 3 cm above the aortic bifurcation. Twenty mg of lidocaine was injected intra-arterially to minimize spasm. A postangioplasty angiogram was performed 10 minutes after the last balloon inflation with the animal positioned exactly as described above. A 1-cm grid was positioned at the level of the femoral artery to permit calculation of the actual luminal diameter by providing correction for magnification. Blood was drawn for a PTT estimation. The angioplasty catheter was then removed, the proximal right carotid artery ligated with 3.0 silk and the wound sutured with a 3.0 vicryl subcuticular stitch. In animals maintained for 28 days after balloon angioplasty \(n=36\), angiography was repeated via the left carotid artery using the above technique, immediately prior to killing.
Sacrifice and Pressure Perfusion

Rabbits were sacrificed 2–24 hours after the angioplasty in the acute group (n=34) and 28 days after in the chronic group (n=36). Through a vertical lower abdominal incision, the distal descending aorta was isolated, tied off proximally, and a perfusion cannula was inserted into the distal segment. Care was taken to position the cannula above the aortic bifurcation. The distal arterial tree was flushed with 50 ml saline followed by fixation of the vessels at in vivo dimensions with 100 ml of 3%-glutaraldehyde solution infused over 15 minutes at 100 mm Hg. Perfusion was at room temperature (22°C). Once the perfusion was started, the animals were killed with an overdose of Nembutol (3 ml sodium pentobarbital i.v., 65 mg/ml, Anthony Products Co). A 5–6 cm segment of femoral artery was excised bilaterally. The proximal and distal ends were marked with silk sutures of different lengths. The tissue was preserved in 3% glutaraldehyde for light microscopy.

Data Analysis

Angiography. The angiograms were read by two blinded observers using electronic digital calipers (Brown & Sharpe Digit-Cal Plus, model 599-571-3). The minimum diameter of the lesion before and after angioplasty was measured by each observer. Acute angiographic success was defined as a greater than 20% increase in luminal diameter.

Restenosis was defined as a greater than 50% loss of initial gain. In 29 of 70 animals (46 vessels), the mean diameters of the vessel segments proximal and distal to the stenosis and the in vivo diameter of the inflated balloon (filled with contrast) at the site of maximal stenosis were measured and expressed as the balloon-to-vessel ratio. This measurement was not made in the remaining vessels because of technical considerations.

Light Microscopy. Thirty-eight acute and 41 chronic angioplastied vessels were prepared for light microscopy. These were cut in serial 4–6-mm sections from the proximal to the distal end and color-coded to identify their relative location to one another. Duplicate slides were stained with hematoxylin and eosin or Richardson’s combination of Verhöff’s elastic and Gomori’s trichrome stains. Specimens were interpreted by two blinded observers.

The analysis consisted of both a descriptive histologic and quantitative morphometric evaluation. The descriptive assessment noted the following: the presence of thrombus in the lumen; the characteristics of the response of the vessel to angioplasty; the presence and site of dissection; the condition of the media with respect to inflammatory cells, dissection, necrosis, and neovascularization. Medial necrosis was graded as: 1+ for mild with focal nuclear degeneration, 2+ for moderate with patchy acellular areas, and 3+ for severe with extensive but patchy vessel-wall necrosis. The integrity of the internal elastic lamina and tunica adventitia were evaluated.

Morphometric analysis. Only vessels from animals killed 28 days after angioplasty were analyzed morphometrically (n=52). Morphometric analysis was not performed on vessels harvested early because the geometric changes immediately after angioplasty made this very difficult. The section with the smallest luminal diameter from each vessel was used. Sections not having a good cross-section or containing preparation artifacts such as folds (n=1) and occluded vessels (n=7) were not analyzed. Morphometric analysis was performed with a digitizing tablet and an electronic cursor (Kurta series one), linked to a IBM XT microcomputer. A drawing tube attached to a microscope was positioned over the digitizing tablet, thereby enabling the examiner to simultaneously visualize the histologic section and the cursor on the digitizing tablet. The cursor was equipped with a cross hair, allowing for precise measurements. The digitizing tablet was calibrated using a scientific grade ruler. The cross-sectional area (mm²) of the lumen, intima, and media was obtained by tracing the diameters of the lumen and the internal and external elastic laminae. The digitized data were stored and analyzed using a commercial software package (PRODESIGN II). Each measurement was made in duplicate and the average recorded.

Statistical analysis. Data were expressed as the mean±1 SD. The angiographic data within each group were analyzed using the paired Student’s t test and intergroup comparisons performed using the unpaired Student’s t test with the Bonferroni correction for multiple groups.

The acute angiographic success, acute histology, and restenosis rates were analyzed using the twotailed Fisher exact test. The morphometric results were compared using the unpaired Student’s t test. A p value of less than 0.05 was considered significant.

Results

Angiography—Acute

The change in angiographic diameters before and 10 min after angioplasty for the different subsets (groups 1–4) are illustrated in Figure 2. The baseline vessel diameters for each group were similar. There was a significant increase in luminal diameter for all groups following balloon dilatation (group 1: 1.3±0.2 to 1.8±0.3; group 2: 1.2±0.2 to 2.0±0.6; group 3: 1.2±0.3 to 1.5±0.5; group 4: 1.3±0.3 to 1.7±0.3 mm; p<0.01 for all groups). Acute angiographic success, arbitrarily defined as a >20% increase in luminal diameter, was highest in the high inflation pressure groups 2 (32 of 36 [89%]) and 4 (16 of 19 [84%]) versus the low inflation groups 1 (25 of 35 [71%]) and 3 (nine of 17 [53%]), p<0.05 (Table 1), groups 2 and 4 versus 1 and 3.

Primary angioplasty failure (<20% increase in luminal diameter) occurred in 25 arteries. The major-
ity (76%) of these failures occurred with low (5 atm) inflation pressure angioplasty. Acute vessel closure occurred in one (6%) group 3 (appropriate size balloon, low inflation pressure) and in two (6%) group 2 (oversized balloon, high inflation pressure) arteries but was not seen in the other groups. A representative series of angiograms before and after balloon angioplasty are shown in Figure 3. The example demonstrates acute closure with a dissection following angioplasty of the right femoral artery in a rabbit randomly selected to survive 28 days. At 28 days (not illustrated), the occluded vessel was patent. This suggests that the occlusion was due to an associated occlusive thrombus that subsequently lysed, severe spasm that reversed spontaneously, an intimal flap, or a combination of two or all three of these processes. The balloon-to-vessel ratio was measured in a total of 46 vessels (group 1, 11; group 2, 11; group 3, 12; group 4, 12) (Table 1). A 3.0-mm balloon resulted in oversizing (i.e., ratio, >1.3:1)

![Graph](image)

**Figure 2.** Individual angiographic diameters before (pre) and 10 minutes after (post) angioplasty for the four experimental groups are illustrated. Panel A: group 1: 3.0-mm balloon and 5-atm inflation pressure. Mean minimum intraluminal diameter increased from 1.3±0.2 to 1.8±0.3 mm. Panel B: group 2: 3.0-mm balloon and 10-atm inflation pressure. Mean minimum intraluminal diameter increased from 1.2±0.2 to 2.0±0.6 mm. Note: Acute closure occurred in two arteries (6%). Panel C: group 3: 2.5-mm balloon and 5-atm inflation pressure. Mean minimum intraluminal diameter increased from 1.2±0.3 to 1.5±0.5 mm. One vessel closed abruptly (6%). Panel D: group 4: 2.5-mm balloon and 10-atm inflation pressure. Mean minimum intraluminal diameter increased from 1.3±0.3 to 1.7±0.3 mm. All differences were significant at the p<0.01 level.

### Table 1. Influence of Balloon Size, Inflation Pressure and In Vivo Balloon to Vessel Ratio on Angiographic Outcome

<table>
<thead>
<tr>
<th>Group</th>
<th>Balloon size (mm)</th>
<th>Inflation pressure (atm)</th>
<th>In vivo size (mm)</th>
<th>Balloon to Vessel ratio</th>
<th>Acute* success (%)</th>
<th>Restenosis (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>5</td>
<td>3.0±0.1</td>
<td>1.5±0.1</td>
<td>23/35 (66)</td>
<td>14/20 (70)</td>
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<td>(n=11)</td>
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<tr>
<td>2</td>
<td>3.0</td>
<td>10</td>
<td>3.3±0.1</td>
<td>1.6±0.1</td>
<td>32/36 (89)</td>
<td>11/16 (69)</td>
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<td></td>
<td></td>
<td></td>
<td>(n=11)</td>
<td></td>
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<tr>
<td>3</td>
<td>2.5</td>
<td>5</td>
<td>2.4±0.1</td>
<td>1.1±0.1</td>
<td>9/17 (53)</td>
<td>5/10 (50)</td>
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<td></td>
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<td></td>
<td>(n=12)</td>
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<tr>
<td>4</td>
<td>2.5</td>
<td>10</td>
<td>2.5±0.1</td>
<td>1.1±0.1</td>
<td>16/19 (84)</td>
<td>5/10 (50)</td>
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<td></td>
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<td>(n=12)</td>
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*In vivo size, balloon size measured in vivo; Acute success, >20% increment in luminal diameter; Restenosis, >50% loss of initial gain.

*p<0.05, groups 2 and 4 vs. 1 and 3.
FIGURE 3. A series of angiograms before (pre) and 10 minutes after (post) bilateral femoral artery balloon angioplasty using a 3.0-mm balloon and a 5- or 10-atm inflation pressure. Panel A: Preangioplasty angiogram: Bilateral focal femoral atherosclerotic lesions are arrowed; Ao, aorta. Panel B: Three-millimeter balloon was inflated to 10 atm across right femoral stenosis. Panel C: Five atmospheres inflation across the left femoral stenosis. Note waist in balloon at site of maximal narrowing. Panel D: Ten-minute postangioplasty angiogram with acute vessel closure at site of high pressure angioplasty. Left femoral artery angioplasty was successful.

independent of inflation pressure (mean balloon-to-vessel ratio, 1.5±0.1). A 2.5-mm balloon was appropriately sized even at high inflation pressure (mean, 1.1±0.1). The balloon size achieved in vivo corresponded to the manufacturer’s predetermined balloon diameter for groups 1, 3, and 4, but overdis- tention occurred in group 2 (oversized balloon, high inflation pressure).

The influence of balloon size, inflation pressure, and in vivo balloon-to-vessel ratio on the angiographic success in the four groups is summarized in Table 1. This illustrates that the high inflation groups, independent of balloon size, achieved the best initial results.

Histologic Changes Early After Angioplasty (Table 2 and Figure 4)

The acute histologic consequences following angioplasty in the four groups are summarized in Table 2. At 2–24 hours after balloon dilatation and despite intraprocedural anticoagulation (PTT 2–2.3×control), 13 of 21 (62%) high pressure (groups 2 and 4) versus three of 17 (18%) of the low pressure (groups 1 and 3) vessels had mural thrombi present at histologic exam-

<table>
<thead>
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<th>TABLE 2. Acute Histologic Consequences Following Angioplasty</th>
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<td>Groups</td>
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<tr>
<td>Group 1 (n=11)</td>
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<td>Group 2 (n=13)</td>
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<tr>
<td>Group 3 (n=6)</td>
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<td>Group 4 (n=8)</td>
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* p<0.01 groups 1 and 3 vs. 2 and 4; † p<0.05 groups 1 and 3 vs. 2 and 4 for 2+ and 3+ medial necrosis.

Group 1, 3.0 mm/5 atm; group 2, 3.0 mm/10 atm; group 3, 2.5 mm/5 atm; group 4, 2.5 mm/10 atm. Medial necrosis: 1+, mild with focal nuclear degeneration; 2+, moderate, with patchy areas of acellularity; 3+, severe with extensive circumferential necrosis of the wall. Note, in group 3 there was one vessel without evidence of medial necrosis.
In the setting of vessel-wall dissection, which was more common in the high pressure groups, \( p < 0.01 \). Complete thrombotic occlusion was seen in one high pressure group. Moderate to severe necrosis of the media (2+ and 3+) was more common in the vessels subjected to high pressure angioplasty 20/21 (95%) compared to low pressure vessels 8/17 (47%) \( p < 0.05 \). An inflammatory infiltrate of neutrophils and lymphocytes was seen in all groups. Seven of the 25 arteries with primary angioplasty failure (>20% increase in luminal diameter) had acute histologic evaluation. In this group, the incidence of thrombus, dissection, and medial necrosis was 1/7 (14%), 4/7 (57%), and 5/7 (71%), respectively.

Vessel-wall damage following angioplasty was more extensive in high inflation pressure angioplasty with a significantly higher incidence of dissection, mural thrombi, and extensive medial necrosis.

**Angiography—Chronic**

Thirty-six rabbits underwent chronic evaluation at 28 days. The individual angiographic diameters before, after, and at 28 days after angioplasty for the four groups are illustrated in Figure 5. In each group, the initial gain after angioplasty was lost at 28 days (group 1: oversized balloon, low inflation pressure, 1.4±0.2 before angioplasty to 1.8±0.3 after to 1.1±0.7 at 28 days; group 2: oversized balloon, high inflation pressure, 1.2±0.2 before to 2.0±0.7 after to 1.3±0.6 at 28 days; group 3: appropriate size balloon, low inflation pressure, 1.2±0.4 before to 1.4±0.5 after to 1.1±0.6 at 28 days; group 4: appropriate size balloon, high inflation pressure, 1.4±0.2 before to 1.7±0.2 after to 1.5±0.3 mm; \( p < 0.01 \), 28 days versus after in groups 1, 2, and 4). Total vessel occlusion was seen in group 1: 2/20 (10%), group 2: 2/16 (12%), and group 3: 2/10 (20%). All group 4 vessels were patent. A representative series of angiograms at baseline, 10 minutes after, and 28 days after angioplasty are shown in Figure 6.

The restenosis rates, defined as a greater than 50% loss of initial gain, were similar in all groups (group 1: 14/20 [70%]; group 2: 11/16 [69%]; group 3: 5/10 [50%]; and group 4: 5/10 [50%]; \( p = NS \)). Using the Fisher exact test, groups 1 and 2 (oversized balloon) combined were not different from groups 3 and 4 combined (appropriately sized) \( p < 0.16 \), although there was a trend in favor of a higher restenosis rate. In the subset (from all four groups) with primary angiographic failure evaluated at 28 days \( n = 17 \), the mean minimal luminal diameter remained unchanged (1.3±0.3, \( p = NS \) vs. before and after angioplasty diameters). In the control nonangioplastied group \( n = 8 \), the angiographic diameter remained unchanged (1.3±0.2 after 1 month on the atherogenic diet versus 1.3±0.1 28 days later).

**Histologic Changes 28 Days After Angioplasty (Figure 7)**

Of the 51 vessels (group 1, 17; group 2, 15; group 3, 9; group 4, 10) examined histologically at 28 days after balloon angioplasty, eight vessels (16%) were totally occluded by foam cells and patchy fibrosis. Nonoccluded vessels had a concentric, multilaminated neointima composed of smooth muscle cells, foam cells, and a fibrotic extracellular matrix. Occasionally the fibrosis was in the form of a crescentic
FIGURE 5. Individual angiographic diameters before (pre), after (post), and 28 days after angioplasty. Panel A: group 1: 3.0-mm balloon and 5-atm inflation pressure. The mean intraluminal diameters before, after, and at 28 days were 1.4±0.2 mm, 1.8±0.3 mm, and 1.1±0.7 mm (p<0.001, *before vs. after, **28 day vs. after). Ten percent of arteries (two of 20) were totally occluded. Panel B: group 2: 3.0-mm balloon and 10-atm inflation pressure. Mean intraluminal diameters before, after, and at 28 days were 1.2±0.2 mm, 2.0±0.7 mm, and 1.3±0.6 mm (p<0.001, *before vs. after, **28 day vs. after). At 28 days, two of 16 arteries were totally occluded. A single artery was totally occluded immediately after angioplasty but was patent at 28 days (see Figure 3). Panel C: group 3: 2.5-mm balloon and 5-atm inflation pressure. Mean diameters before, after, and at 28 days were 1.2±0.4 mm, 1.4±0.5, and 1.1±0.6 mm (p=NS, before vs. after, 28 day vs. after, p<0.05). Two of 10 arteries were totally occluded at chronic evaluation. Panel D: group 4: 2.5-mm balloon and 10-atm inflation pressure. Mean intraluminal diameters before, after, and at 28 days were 1.4±0.2 mm, 1.7±0.2 mm, and 1.5±0.3 mm (p<0.01, *before vs. after, **28 day vs. after). No vessels in this group were totally occluded.

scar (Figure 7). In some vessels, areas of neovascularization and patchy areas of reduplication of the internal elastic lamina were present. The effect on the media was variable, with patchy fibrosis in some vessels and extensive scar formation and distortion of the normal architecture in others. In contrast to

the acute histological pattern, mural thrombi were not seen. The nonangioplastied atherosclerotic control arteries (n=8) had predominantly concentric neointimal plaque and an intact internal elastic lamina in the majority. The media contained foam cells interspersed with smooth muscle cells.

**Morphometric Analysis**

Morphometric analysis is summarized in Figure 8. There was no significant difference in the cross-sectional area of the lumen or media among groups or versus control.

The most noteworthy finding was the significant increase in the degree of intimal hyperplasia between controls (0.5±0.2 mm²) and all other groups (group 1: 0.9±0.5 mm², group 2: 1.7±0.9 mm², group 3: 1.0±0.6 mm², group 4: 1.2±0.6 mm²; p<0.05). This was most marked with the combination of an oversized balloon and high inflation pressure (group 2, p<0.001). In the subset of vessels with primary angioplasty failure, despite no evidence of a decrease in luminal diameter by angiography, the degree of intimal hyperplasia was significantly larger than control (0.9±0.5 vs. 0.5±0.2 mm², p<0.05).

**Serum Cholesterol Levels**

The mean serum cholesterol levels at baseline were similar in all groups (44±8 mg%) and rose significantly at the time of angioplasty (1,146±238 mg%, p<0.0001). Levels in animals with chronic total occlusions (1,212±350 mg%) were similar to the population as a whole.

**Discussion**

We had four major findings from this study. 1) In absolute terms all angioplasty groups had a significant increase in luminal diameter immediately following balloon angioplasty. 2) Acute angiographic success, defined as a greater than 20% increase in luminal diameter, was more frequent in the high inflation balloon angioplasty groups (groups 2 and 4) but was achieved at the expense of more extensive vessel-wall damage. 3) Restenosis, defined as a greater than 50% loss of initial gain, was similar in all groups although there was a trend (not statistically significant) toward a higher incidence with oversized balloons (groups 1 and 2). 4) All protocols caused intimal hyperplasia, being most marked when the most traumatic procedure, a combination of an oversized balloon and high inflation pressure (group 2), was used. The implication of the findings from
this experimental study is that balloon size should be matched to vessel lumen to minimize the degree of intimal hyperplasia.

It is noteworthy that mural thrombus occurred exclusively in the setting of wall dissection, which was more common in the high inflation groups, suggesting that an important mechanism by which balloon angioplasty causes luminal enlargement was also a potent stimulus for thrombus formation. Thrombus formation, in turn, may be a factor in the augmented proliferative response in the arterial wall causing excessive intimal hyperplasia and restenosis. Intimal thickening is probably driven by release of platelet-derived growth factor and by the hyperlipemic blood containing elevated levels of low density lipoprotein, which both stimulate smooth muscle cell migration and proliferation. In addition, the smooth muscle cells in culture have been shown to undergo a phenotypic change from being primarily contractile to predominantly secretory-type cells capable of synthesizing and secreting many of the components of the excessive extracellular matrix in the lesion. Thus, it is possible that these factors combine to cause intimal hyperplasia in all groups, with the most rapid acceleration of the process in group 2 (oversized balloon, high inflation pressure).

The histologic correlate of the consequence of angioplasty has been described in previous animal studies and also in a limited number of reports on humans. However, we believe that this represents the first report correlating histology with specific technical factors. The 28-day specimens from this study (Fig. 7) are similar to the human experience. The major difference is that human atherosclerosis generally does not contain foam cells at the time of balloon angioplasty. Austin et al. described three patients dying at 5, 17, and 62 days after coronary angioplasty. He reported that, at 62 days after angioplasty, circumferential intimal proliferation of smooth muscle cells, which extended into intimal cracks, was seen. A similar proliferative response has been reported by Essed et al., 5 months after coronary angioplasty.

Waller et al., in three necropsy patients, 80, 90, and 150 days after a clinically successful coronary angioplasty, found no morphologic lesions attributable to the angioplasty. However, this may be a function of the variability of the degree of arterial injury at time of angioplasty. Kohchi et al., in 20 coronary artery sites from nine patients, found a variable histologic pattern consisting of tears into the intima and media with repair by organized thrombus and of smooth-muscle cell growth.

The high incidence of mural thrombus, seen early after high inflation pressure angioplasty, occurred despite anticoagulation with heparin in a dose usually considered within the therapeutic range. The relation between thrombosis, anticoagulation, and angioplasty has recently been addressed by Heras et al., who emphasized the importance of adequate heparin dose during the procedure. They found that platelet deposition after deep arterial injury angioplasty was inversely related to the amount of heparin administered per kilogram of body weight per unit of time. They required 3.1 units/kg/min to prevent thrombosis in the normal carotid artery of the pig. We used a bolus dose of 100 units/kg immediately prior to angioplasty, which may not have been sufficient to achieve a local antithrombotic effect.

The Atherosclerotic Rabbit as a Model for Human Angioplasty

Any one animal model of atherosclerosis has limitations. We, in this paper, and others have demonstrated that experimental atherosclerosis can be consistently induced in the New Zealand White rabbit by endothelial injury followed by a high cholesterol (2%-cholesterol, 6%-peanut-oil) diet. Faxon et al. used balloon denudation of the endothelium or an indwelling catheter technique to induce injury. These techniques produced different histopathologic types of atherosclerotic lesions. These included predominantly foam-cell lesions with balloon injury and eccentric mixed fibrous, foam lesions, or both with the indwelling catheter method. Our technique, originally described by LeVeen et al., involved air-desication injury of the femoral artery followed by an atherogenic diet for 1 month resulting in a generally concentric, mixed fibrocellular lesion. Although the diet induced very high serum levels of cholesterol (1146±238 mg-%), and the atheromatous lesions differ from advanced human atherosclerosis in that necrosis, fibrosis, and calcification are absent, there are many attractive features of this model. These include the rapid and reliable development of stable atherosclerotic lesions that can be assessed precisely by the preangioplasty angiogram and, therefore, are suitable for angioplasty, with similar acute and chronic complications, as documented after human angioplasty.

Implications

Although it may be difficult to extrapolate the results of this experimental study to long-standing human atherosclerosis because of differences in the composition and complexity of the disease, it is nonetheless in agreement with the clinical study from Emory advocating the avoidance of oversized balloons for angioplasty. This recommendation is primarily based on the observation that the immediate complication rate was unacceptably high. The danger of balloon oversizing is emphasized by our study.

In view of the disappointing results yielded by clinical trials of pharmacologic agents directed at platelet deposition, thrombosis, and vascular spasm, the exception being the fish oil study in patients undergoing coronary angioplasty, it would seem reasonable to approach the prevention of restenosis by more aggressively inhibiting early throm-
bus formation with more effective antiaggregation agents and by facilitating clot lysis with more potent thrombolytic agents\textsuperscript{60–66} during the vulnerable period following balloon angioplasty. The recent finding of a lower restenosis rate, after successful emergent coronary angioplasty in the setting of streptokinase therapy for acute myocardial infarction,\textsuperscript{67} and the fish oil study (antiplatelet agent),\textsuperscript{28} demonstrating lower rates of restenosis after angioplasty, support this view. Also evident from this study is the observation that all four angioplasty protocols caused, to a varying degree, an acceleration of intimal hyperplasia. The initial success achieved with the most traumatic combination of the oversized balloon and high inflation pressure was more than offset by the increase in intimal hyperplasia. The key to the prevention of restenosis ultimately rests with the inhibition of cell migration and proliferation.

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