Vascular Reactivity After Balloon Angioplasty in an Atherosclerotic Rabbit

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Alterations in vessel wall reactivity (VR) at or adjacent to the dilation site after balloon angioplasty (BA) may vary according to the inflation protocol and the time after angioplasty and may influence outcome. In 64 atherosclerotic rabbit femoral arteries, we evaluated VR after BA with intravenous ergonovine (ERGO) (40 \( \mu \)g/min for 5 minutes) and intra-arterial nitroglycerin (NTG) (2,500 \( \mu \)g single bolus) 24–72 hours and 28 days after BA. Comparisons were made with atherosclerotic, nonangioplastied, age-matched controls. BA was standardized to three 1-minute inflations, each 1 minute apart. For each balloon size, 2.5- (appropriate size) or 3.0-mm (oversized) vessels were allocated to either 5 or 10 atm inflation pressure. For the analysis, four groups were compared: Group 1, 3.0/5; group 2, 3.0/10; group 3, 2.5/5, and group 4, 2.5 mm/10 atm. Angiographic diameters were measured at, proximal, and distal to the lesion at baseline, 10 minutes after ERGO, and 5 minutes after NTG. Angiograms were measured with electronic calipers by two blinded observers. All segments of control vessels vasoconstricted to ERGO and vasodilated to NTG (\( p < 0.05 \) versus baseline), indicating a normal response. At 24–72 hours after dilatation, the angioplasty sites for all inflation pressure/balloon size combinations were not responsive to either ERGO or NTG. All segments distal to the dilatation sites vasoconstricted to ERGO and dilated to NTG (\( p < 0.05 \) versus baseline), indicating a normal response. Proximal segments of vessels dilated with a 2.5-mm balloon (appropriate size) responded positively to both stimuli (\( p < 0.05 \)). Those vessels dilated with a large balloon (3.0 mm) were nonreactive in the segment proximal to the angioplasty site. Twenty-eight days later angioplasty sites dilated with a 2.5-mm balloon (appropriately sized) regained reactivity; however, segments dilated with a large balloon (3.0 mm) remained unresponsive. All proximal segments, including those from vessels dilated with a large balloon, reacted positively. All distal segments reacted appropriately. Restenosis rates were not different between the over- and appropriately sized balloon groups. These data demonstrate that immediately after angioplasty, vessels lose reactivity at the dilatation site. Those vessels dilated with the smaller-size balloon (2.5 mm) regained reactivity. For large balloons, reactivity is not regained at 28 days. For segments proximal to the site of dilatation, transient loss of reactivity is seen only when a large balloon is used. Thus, acute closure originating at the site of dilatation is not a result of spasm. In addition, no connection was found between restenosis rates and the ability of the dilatation site to react to ERGO and NTG. Finally, the vessel proximal, and especially distal, to the site of angioplasty remains highly reactive, and spasm originating at those sites may contribute to acute closure. (Circulation 1990;82:1790–1801)

The major limitations of balloon angioplasty are acute closure (approximately 4%) and restenosis (30–40% at 3–6 months).1–3 Although the mechanism of each of these processes remains unresolved, there is evidence that vaso- spasm plays a role. In patients undergoing coronary angioplasty, it is estimated that arterial spasm occurs acutely in 3–5% of cases.4 Supporting this observation is the work by Sanders,5 who noted that within 30 minutes of coronary angioplasty there was a decline of 16–62% of the intraluminal diameter at the site of angioplasty, a finding consistent with the work of Fischell et al.6 This phenomenon probably results from vascular spasm but could also be from a dissec-
tion, intimal flap, thrombus/vascular recoil, or a combination of these processes. These factors may all play a role in acute occlusion in the period immediately after angioplasty.7–10

The propensity to develop spasm may not be limited to the period very early after angioplasty.11–13 Quyumi et al12 using an ergonovine (ERGO) infusion, produced coronary spasm at the site of angioplasty in five of 14 patients between 6 and 20 weeks after angioplasty. The relation between provoked spasm and restenosis in patients undergoing coronary angioplasty was studied by Bertrand et al.13 The presence of ERGO-induced coronary spasm at 6 months was associated with a higher incidence of restenosis.

Vascular reactivity after angioplasty may vary with time. In an in vitro system consisting of segments of arterial rings from a nonatherosclerotic rabbit, Consigny et al14 observed arterial paralysis immediately after angioplasty with reversal 28 days later, suggesting that the vessel wall's lost ability to constrict may be transient. This phenomenon has been noted by others.15

The tendency to develop spasm may relate to the degree of damage to the media of the vessel and thus indirectly to dilatation pressure and balloon size. Castaneda-Zuniga et al16,17 and Zollikofer et al18,19 have demonstrated in animal studies that a major component of the mechanism of successful angioplasty requires stretching of the vessel wall, with subsequent medial damage. This phenomenon may prevent the vessels from responding to vasoconstricting and dilating stimuli. Autopsy studies confirm these findings.20–22 Moore23 further advanced this hypothesis by postulating that incomplete medial destruction leaves the vessel vulnerable to vascular spasm, a response that may be mediated through platelet/arterial wall interaction24,25 or an imbalance of endothelium-derived vasoconstricting and dilating agents such as endothelin26 and endothelium-derived relaxing factor.27

We hypothesize that angioplasty does alter vascular reactivity and that it may be influenced by balloon size, inflation pressure, and time after dilatation. It is also probable that the segments immediately proximal and distal to the angioplasty site act differently compared with the angioplasty site itself. Therefore, this study will evaluate vasomotor reactivity to the direct smooth muscle constrictor ERGO and the vasodilator NTG after balloon angioplasty in an atherosclerotic rabbit model. Quantitative evaluations will be made in vivo by angiography and, at, proximal, and distal to the angioplasty site. Balloon inflation pressures at 5 and 10 atm using either a 2.5- (appropriately sized) or a 3.0- (oversized) mm balloon will be compared. These sizes and inflation pressures are within the range used in patients. Observations are reported at 24–72 hours and 28 days after angioplasty. Comparisons were made with histology of the vessel wall.

### Methods

The experimental protocol is outlined in Figure 1.

### Induction of Femoral Atherosclerosis

In 8 10-lb New Zealand White male rabbits, femoral atherosclerosis was induced by endothelial damage with air desiccation followed by a high-fat diet.28 To induce endothelial injury, a 1–2-cm segment of femoral artery 1 cm below the inguinal ligament was isolated and secured between airtight silk ligatures. Local spasm was prevented by topical administration of 0.5 ml 2% lidocaine (Anthocaine injection, 2%). The isolated segment was cannulated with a 27-gauge needle. A posterior vent was created by needle puncture. The segment was then flushed with 1 ml saline. Endothelial injury was induced with desiccated nitrogen gas at a flow rate of 80 ml/min for 8 minutes. Then the ligatures were removed and hemostasis achieved by local pressure. The proximal and distal edges of each damaged segment were demarcated with metal clips (Hemoclip, Edward Weck & Co., Research Triangle Park, N.C.) applied to the adjacent muscle. The skin was closed with a running 4.0 vicryl subcuticular suture. Then each rabbit was placed on a 2% cholesterol and 6% peanut oil diet (Dyets Inc., Bethlehem, Pa.) for 28 days. After this time, normal rabbit chow was resumed. Data collected from separate animals treated with the described diet had initial total cholesterol levels at diet inception of 44±8 mg%. Levels increased to 1,146±238 mg% at 28 days.

### Angioplasty

The rabbits were anesthetized with 35 mg/kg i.m. ketamine and 5 mg/kg i.m. xylazine and maintained with intravenous ketamine and xylazine in an mg/kg ratio of 8:1. Rabbits were positioned in a perspex brace in the supine posture, with the hind legs externally rotated and abducted and the knees in full extension. The position was standardized to ensure comparable angiograms. To isolate either carotid artery, a midline incision in the neck was made. After vessel ligation (cephalad), a 4F introducer was advanced (through an arteriotomy) to the junction of the carotid and aortic arch. Through this instrument, a 0.014 in. USCI veriflex guide wire was then advanced under fluoroscopic guidance to the distal abdominal aorta. Thereafter a Medi-Tech (Mansfield, Mass.) 2.5 or 3.0–2/4.0/120 polyethylene balloon angioplasty catheter was positioned with an over-the-wire catheter exchange. Animals were anticoagulated with 250 IU intra-arterial heparin. Incorporated in our standard protocol, arterial spasm was attenuated with 1 ml 2% lidocaine infused into the distal abdominal aorta. A baseline cut-film angiogram of the distal aorta, iliac, and femoral arteries was obtained by hand injection of 3–6 ml Renografin 76 (diatrizoate meglumin and diatrizoate sodium injection USP, Squibb, New Brunswick, N.J.). The 0.014-in. veriflex guide wire was then advanced.
across the femoral stenosis, and the balloon catheter was positioned with fluoroscopic guidance. Two balloon sizes (3.0 mm and 2.5 mm) were used. Lesions for each balloon were randomly assigned to either 5 or 10 atm inflation pressure. Thus, a catheter change was prevented. Four angioplasty protocols were used: Group 1=3.0 mm/5 atm, group 2=3.0 mm/10 atm, group 3=2.5 mm/5 atm, and group 4=2.5 mm/10 atm. The relevant balloon pressures were achieved using an Indeflator (Advanced Cardiovascular Systems, Inc, Mountain View, Calif.). Inflations comprised three 1-minute periods, with 1-minute intervals between inflations. The position and size of the balloon in vivo was verified by a cut-film radiograph. This radiograph was subsequently used to precisely define the site of angioplasty. One milliliter of 2% lidocaine was selectively administered into the corresponding iliac artery at completion of the dilatations. When it was possible to perform bilateral angioplasties, the same catheter (therefore the same balloon size) was used. However, dilatation pressures alternated between 5 and 10 atm. An angiogram after the angioplasty was obtained 10 minutes after the final balloon inflation. After completion of the procedure, the carotid artery was ligated with 4.0 silk and the skin incision closed with a running subcuticular 3.0 vicryl suture.

**Provocative Testing With Ergonovine and Nitroglycerin**

Reactivity studies were performed 24–72 hours and 28 days after angioplasty. Controls were age-matched and consisted of atherosclerotic, nonangioplasted vessels. Twenty-six vessels from 21 rabbits were evaluated 24–72 hours after angioplasty. Sixteen rabbits had unilateral angioplasty because the contralateral side was totally occluded and five had bilateral angioplasties. At 28 days, 20 rabbits had unilateral and nine had bilateral reactivity studies for a total of 38 vessels. Rabbits were anesthetized and positioned (as previously described) for the initial angioplasty procedure. By means of an arteriotomy, a 4F introducer was placed into the proximal carotid artery and an angioplasty catheter positioned in the distal abdominal aorta. Anticoagulation was achieved with 250 IU intra-arterial heparin. A baseline angiogram was obtained, and no lidocaine was administered. The response to ERGO was ascertained by a previously determined dose to induce 30% angiographic narrowing of the femoral artery in normal rabbits. Forty micrograms of ERGO was infused intravenously each minute for 5 minutes (total dose of 200 μg ERGO). Angiography was repeated 10 minutes after the last dose of ERGO. After 5 minutes, NTG was intra-arterially administered in a 2,500-μg bolus, and angiography was repeated 5 minutes later. Angiographic measurements were made at the level of the femoral head (proximal segment) and at the site of the angioplastied or nonangioplastied lesion as well as distal to the dilatation site or control lesion (midfemur). Measurements were also made of the abdominal aorta (two vertebrae above bifurcation) (see Figure 2).
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FIGURE 2. Depiction of series of representative angiograms. In this case, balloon inflation pressure was 5 atm for right and 10 atm for left femoral artery. Balloon was 2.5 mm in diameter. Top of each panel faces cephalad. Panel A: Preangioplasty angiogram. AO, aorta. Arrows point to discrete stenoses produced by air dessication injury and high-lipid diet. Note caliber of proximal and distal vessels. Panel B: Angiogram after angioplasty. Arrow 1 points to lesions that were dilated. Arrow 2 points to spasm distal to angioplasty site. Panel C: Angiogram obtained 28 days after angioplasty and 10 minutes after administration of ergonovine. Note the intense vasospasm proximal and distal to and at angioplasty site. Panel D: After administration of nitroglycerin. Intense vasospasm is reversed. This was best seen in right femoral artery, which was dilated to 5 atm.

Animal Welfare
On completion of the provocative testing, the animal was killed with an overdose of intravenous Nembutol (sodium pentobarbital injection, 65 mg/ml; Abbott Laboratories, North Chicago, Ill.). The care and disposal of the rabbits accorded with the specifications of the Animal Welfare Act. All procedures were performed under general anesthesia as described and with sterile surgical techniques. Antibiotic coverage for all (except terminal) procedures was achieved with intravenous gentamicin (1 mg/kg).

Data Analysis
Angiograms. Angiograms were read by two observers in a blinded fashion. The intraluminal diameter of the distal aorta, the proximal and distal femoral artery segments, and the minimum diameter of the atherosclerotic or angioplastied site were measured using electronic calipers (Model 599-571-3, Brown and Sharp Digit-Cal Plus, Athol, Mass.). Measurements were corrected for magnification in all angiograms by use of a 1-cm grid placed at the femoral arteries. The correlation coefficient for interobserver variability was 0.94. Intraobserver variability was 0.99 for reader 1 and 0.96 for reader 2. Thus, the average value for the two observers was used for the analysis. Response variation to ERGO or NTG was expressed as the percentage of baseline diameter (mean±1 SEM). Responses within a treatment group were compared using Student's t test with a Bonferroni correction. Vessel-based comparisons were made.

Results
The results are summarized in Table 1.

Control Age-Matched, Nonangioplastied Atherosclerotic Vessels

Initial assessment. The intraluminal diameters before and 10 minutes after the administration of ERGO and 5 minutes after the administration of NTG at the site of focal femoral atherosclerosis and
the adjacent proximal and distal arterial segments for the seven nonangioplastied control vessels are illustrated in Figure 3. The proximal segments (panel A) responded normally to ERGO maleate (vasoconstriction) and NTG (vasodilatation). Baseline, post ERGO, and after NTG intraluminal diameters were 2.25±0.05, 1.86±0.06, and 2.1±0.06 mm, respectively. The arterial segments distal to the atherosclerotic lesion produced the expected normal response (panel C, Figure 3). Intraluminal diameters at baseline, ERGO, and after NTG were 1.82±0.06, 1.45±0.07, and 1.68±0.7 mm, respectively. The lesion showed intact vasomotor reactivity to both ERGO and NTG (panel B, Figure 3). Diameters were 1.28±0.05 at baseline, 1.06±0.08 after ERGO, and 1.30±0.04 after NTG. When expressed as a ratio of the change relative to the before-intervention baseline (intervention:control) the lesion, proximal, and distal segments constricted to 0.83±0.02, 0.83±0.2, and 0.80±0.03 mm of baseline in response to ERGO (p=NS among the three sites). The vessels vasodilated in response to NTG from this constricted state toward baseline, with a ratio after NTG/control of 1.02±0.03 at the focal atherosclerotic site, 0.94±0.02

### TABLE 1. Summary of Results

<table>
<thead>
<tr>
<th></th>
<th>Control (3.0 mm/5 atm)</th>
<th>Group 1 (3.0 mm/10 atm)</th>
<th>Group 2 (2.5 mm/5 atm)</th>
<th>Group 3 (2.5 mm/10 atm)</th>
<th>Group 4 (2.5 mm/10 atm)</th>
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<tr>
<td><strong>Proximal segment</strong></td>
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<td>+</td>
<td>-</td>
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<td></td>
<td>28 days</td>
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<td><strong>Lesion or dilated segment</strong></td>
<td>Acute</td>
<td>+</td>
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<td></td>
<td>28 days</td>
<td>+†</td>
<td>-</td>
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<tr>
<td><strong>Distal segment</strong></td>
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<td>+</td>
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<td></td>
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<tr>
<td><strong>Restenosis</strong></td>
<td>28 days</td>
<td>10/17 (groups 1 and 2 combined)</td>
<td>5/9 (groups 3 and 4 combined)</td>
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+, Reactive; −, nonreactive; Acute, 24–72 hours after angioplasty; 28 days, 28 days after angioplasty.

*Significant for ergonovine but not significant for nitroglycerin.

†Significant for nitroglycerin but not significant for ergonovine.

**FIGURE 3.** This figure summarizes response of control animals to ergonovine (Ergo) and nitroglycerin (Nitro). Panels A, B, and C represent studies that were performed at times corresponding to studies immediately after angioplasty and panels D, E, and F correspond to experimental studies performed 28 days after angioplasty. Panels A and D represent segment proximal to the lesion, panels B and E at lesion, and panels C and F segment distal to lesion. Anticipated response of vasoconstriction to Ergo and vasodilation to Nitro is seen.
at the proximal segment, and $0.93 \pm 0.02$ at the distal segment ($p=NS$ among the three sites).

Thus, arterial responsiveness to both ERGO and NTG at the atherosclerotic lesion and at the proximal and distal sites were similar and showed the expected vasoconstricting and dilating response.

At 28 days after the initial evaluation (Figures 3D, 3E, and 3F). Five vessels were tested 28 days after initial testing to evaluate temporal influence on reactivity. In general, the response at the focal atherosclerotic site and the adjacent proximal and distal arterial segments were similar to those seen at the initial assessment. One exception was the response at the lesion to ERGO, which showed a vasoconstrictive trend that was not statistically significant. Thus, any change in vascular reactivity ascribed to angioplasty cannot be explained on the basis of elapsed time.

**Effect of Angioplasty**

All groups had similar lesion diameters (Figure 4) before angioplasty. Immediately after angioplasty, diameters at the angioplasty site were significantly increased in all groups (group 1, $1.31 \pm 0.04-1.61 \pm 0.06$ mm; group 2, $1.28 \pm 0.05-2.3 \pm 0.2$; group 3, $1.2 \pm 0.1-1.7 \pm 0.1$; and group 4, $1.27 \pm 0.04-1.8 \pm 0.1$). Thus, all groups had angiographically successful angioplasties; the difference was statistically significant ($p<0.05$) in groups 1, 2, and 4, and in group 3 there was a trend toward significance.

**Vasomotor Reactivity at 24–72 Hours After Angioplasty**

The 24–72 hour delay was instituted to provide time for residual spasm to resolve after angioplasty spasm. A baseline angiogram was obtained immediately before the administration of ERGO.

At the angioplasty site (Figure 4). The baseline intraluminal diameter before testing was similar to the diameters immediately after angioplasty in all groups (group 1, $1.61 \pm 0.06$ versus $1.6 \pm 0.1$; group 2, $2.3 \pm 0.2$ versus $2.2 \pm 0.3$; group 3, $1.7 \pm 0.1$ versus $1.8 \pm 0.2$; and group 4, $1.8 \pm 0.1$ mm versus $1.8 \pm 0.1$ mm [$p=NS$ for all groups]). In all groups, the dilated segments were unresponsive to both ERGO and NTG.

**Proximal segments (Figure 5).** In vessels dilated with a 3.0-mm balloon (maximum inflated balloon: vessel ratio was $1.35 \pm 0.08$ for group 1 and $1.7 \pm 0.2$ for group 2), the adjacent proximal segments showed no vasoconstricting response to ERGO ($1.6 \pm 0.1$ versus $1.6 \pm 0.1$ for group 1 and $2.2 \pm 0.3$ versus $2.2 \pm 0.3$ for group 2) or vasodilation to NTG ($1.6 \pm 0.1$ versus $1.68 \pm 0.8$ for group 1 and $2.2 \pm 0.3$ versus $2.2 \pm 0.3$ for group 2). Thus, the proximal segments from groups 1 and 2 exhibited behavior similar to the lesioned segments.

Proximal segments from groups 3 and 4 (2.5-mm balloons) had appropriate responses to ERGO and NTG: $1.9 \pm 0.2-1.4 \pm 0.2$ and $2.07 \pm 0.06-1.8 \pm 0.1$ for
ERGO and 1.4±0.2–1.6±0.1 and 1.8±0.1 to 2.1±0.1 for NTG. The balloon:vessel ratios for the 2.5-mm balloon were 1.05±0.02 for group 3 and 1.07±0.04 for group 4.

**Distal segments.** After angioplasty, segmental vasospasm distal to the lesion was seen in all groups. This phenomenon persisted for at least 24–48 hours. Persistence of vasospasm might reflect the continuing deposition of platelets and release of serotonin or the effect of endothelin released from endothelial cells. In all groups, these segments demonstrated further vasoconstriction in response to ERGO, which was then reversed with NTG (group 1, 1.5±0.2–1.1±0.1; group 2, 1.2±0.1–1.0±0.1; group 3, 1.6±0.2–1.4±0.2; and group 4, 1.4±0.1–1.1±0.1 mm; p<0.05). The response to NTG was vasodilation (group 1, 1.1±0.1–1.50±0.08; group 2, 1.0±0.1–1.3±0.1; group 3, 1.2±0.2–1.4±0.2; and group 4, 1.1±0.1 mm–1.5±0.1 mm). Thus these segments demonstrated appropriate vasoreactivity.

In separate experiments, five vessels were administered a saline infusion after ERGO to determine if the vasodilation seen with NTG resulted from attenuation of the effect of ERGO over time. No significant vasodilation was seen with the saline infusion 1.14±0.08 mm after ERGO versus 1.2±0.1 mm after saline (p=NS). Subsequent treatment with NTG resulted in a significant increase in intraluminal diameters from 1.2±0.1 mm after saline to 1.4±0.1 mm after NTG (p<0.006).

**Vasomotor reactivity 28 days after angioplasty—sites (Figure 6).** The restenosis rate by angiography was similar for both balloon sizes. Therefore, no connection was observed between the presence of induced vasospasm and restenosis (Table 1). In vessels dilated with a 3.0-mm balloon (group 1, balloon:vessel ratio 1.5±0.1 and group 2, balloon:vessel ratio 1.7±0.2), the dilated segments remained unresponsive to both ERGO (group 1, 1.3±0.1–1.2±0.1 mm [p=NS] and group 2, 1.8±0.2–1.7±0.2 mm [p=NS]) and NTG (group 1, 1.2±0.1–1.3±0.1 mm and group 2, 1.7±0.2–1.8±0.2 mm [p=NS]). In vessels dilated with a 2.5-mm balloon (group 3, balloon:vessel ratio 1.06±0.03 and group 4, balloon:vessel ratio 1.11±0.03), the dilated segments were reactive to ERGO (group 3, 1.41±0.08–1.21±0.09 mm and group 4, 1.50±0.09–1.38±0.07 mm) and NTG (group 3, 1.21±0.09–1.34±0.07 mm and group 4, 1.38±0.07–1.44±0.08 mm).

**Proximal segments (Figure 7).** The proximal segments of vessels undergoing angioplasty with a 3.0-mm balloon reacted positively to ERGO (group 1, 2.09±0.07–1.81±0.08 mm and group 2, 2.12±0.07–1.83±0.06 mm). Appropriate vasodilation was seen with NTG (group 1, 1.81±0.08–1.99±0.09 mm and group 2, 1.83±0.06–2.00±0.05 mm).
Thus, vasomotor responsiveness, which was lost immediately after angioplasty, was regained at 28 days. For groups 3 and 4, the proximal segments reacted positively to ERGO (group 3, 2.1±0.1–1.68±0.5 mm and group 4, 2.30±0.09–1.87±0.08 mm) and NTG (group 3, 1.68±0.05–1.94±0.09 mm and group 4, 1.87±0.8–2.1±1.0 mm). Thus, for the 2.5-mm balloon, vasomotor responsiveness was similar to that observed 24–72 hours after angioplasty.

**Distal segments.** All distal segments appropriately constricted to ERGO and dilated to NTG. Thus, no change from observations made 24–72 hours after angioplasty was seen (ERGO group 1, 1.8±0.05–1.46±0.06 mm; group 2, 1.86±0.05–1.57±0.04 mm; group 3, 1.83±0.08–1.46±0.08 mm; and group 4, 1.96±0.08–1.6±0.1 mm; NTG group 1, 1.46±0.06–1.61±0.06 mm; group 2, 1.57±0.04–1.79±0.05 mm; group 3, 1.46±0.08–1.79±0.08 mm; and group 4, 1.6±0.1–1.7±0.1 mm).

**Separate Analysis of Unilateral Angioplasties**

An analysis using only those vessels from rabbits with unilateral angioplasties was performed. For studies performed immediately after angioplasty, a nonsignificant trend identical to that described in “Results” was seen. The same type of analysis was performed for data collection at 28 days with similar results. Thus, the phenomenon we describe is vessel and not rabbit specific.

**Histology**

The acute and chronic histological consequences following angioplasty have been previously reported in detail. In summary, vessel wall damage immediately after angioplasty was more extensive using high inflation pressure and an oversized balloon, with an accompanying significantly higher incidence of mural thrombi, dissection, and extensive medial necrosis. The use of an appropriately sized balloon and low inflation pressure made medial necrosis less marked. Figure 8 illustrates acute differences in rabbits from this study. Vessels were prepared as previously discussed. Immediately after angioplasty, all groups failed to react to both ERGO and NTG; however, not all individual vessels did so. The vessel illustrated in panel A reacted normally to both ERGO and NTG. The vessel displayed in panel B was nonreactive. We believe that the difference was due to the degree of damage to the media.

The chronic histological changes included a predominantly concentric, multilaminated neointima comprising smooth muscle cells and some foam cells with a scattered extracellular matrix. Occasionally, fibrosis was seen in the form of a crescentic scar corresponding to a previous dissection. The effects on the media varied. It ranged from patchy scar with normal media to extensive circumferential scarring. The presence of a significant amount of normal...
media was usually associated with a reactive vessel; however, there was a broad spectrum of histological changes. Attempts to rigorously quantify these and correlate changes with vascular reactivity were not successful.

Segments proximal and distal to the dilatation sites were histologically normal.

Discussion

The major findings (given in Table 1) of this study are now enumerated. First, a loss of vasomotor reactivity at the dilatation site was observed 24–72 hours after angioplasty in all experimental groups. Second, at 28 days after angioplasty, vasomotor reactivity was regained at the dilatation site only in the vessels dilated with an appropriately sized (2.5-mm) balloon. Conversely, vessels dilated with a large (3.0-mm) balloon remained nonreactive. Third, the segment adjacent and proximal to the dilatation sites in which an oversized balloon was used also demonstrated a transient loss of vasomotor activity, which was reversed at 28 days after angioplasty. Fourth, the segment adjacent and distal to the dilatation site in all experimental groups, as well as the proximal segments of the appropriately sized balloon groups, responded appropriately on both occasions. Thus, vascular reactivity after balloon angioplasty varies according to site, balloon size, and time after angioplasty. Fifth, the restenosis rate was not different in terms of the balloon sizes used. And last, the presence of a significant amount of intact media was generally necessary for a normal vasomotor response.

Proposed Mechanism for the Permanent and Transient Loss of Reactivity

The mechanism of dilatation by balloon angioplasty is disruption or splitting of the plaque and stretching of the underlying arterial wall to increase the intraluminal diameter of the vessel.\textsuperscript{14,17–19,20–22,30–32} Histologically there is intimal denudation and widespread myocyte necrosis and dehiscence of collagen in the media.\textsuperscript{18,19} The degree of medial change is dependent on the extent of dilatation. When dilated with a 25% oversized balloon, medial changes are confined to the inner one fourth of the artery, as compared with marked changes in over one half of the wall thickness with a 50% oversized balloon.\textsuperscript{19} Biochemical evaluation of vascular smooth muscle cells after balloon angioplasty using determinations of intracellular water content and sodium pump activity also reveals significant smooth muscle damage.\textsuperscript{14}

In an extensive histologic evaluation of the influence of balloon size and inflation pressure on the atherosclerotic vessel wall after angioplasty, we have previously shown that severe circumferential necrosis more commonly occurs using the oversized balloon with low inflation pressure (group 2). However, it is important to emphasize that the degree of medial damage represents spectrum from group 3 (appropriately sized balloon with low inflation pressure, the
FIGURE 8. Photomicrographs of vessel dilated with oversized balloon with low inflation pressure (panel A) and one with high inflation pressure (panel B). Vessel in panel A reacted normally to both ergonovine (Ergo) and nitroglycerin (Nitro). Vessel in panel B was nonreactive. In panel A, luminal surface of neointima (1) has shaggy appearance (2), representing endothelial loss/damage. Elastica interna (3) is grossly intact, and underlying media (4) shows focal areas of nuclear loss (arrow). In other areas (5), media are relatively normal. Hematoxylin and eosin, $\times 250$. In panel B, there is extensive vessel wall injury, with subintimal dissection containing red blood cells (1), fracture of internal elastica (2), and extensive necrosis of media (3). 4, intima. Hematoxylin and eosin stain; original magnification, $\times 250$. 
least) to group 2 (oversized balloon with high inflation pressure, the most). Our histologic examples show that morphologically assessed medial damage at the dilatation site prevents appropriate vasomotor reactivity to both agents immediately and at 28 days following balloon angioplasty (Figure 8). The data we present are consistent with the work of Hackett et al, who demonstrated that the constricting effect of ERGO maleate directly affects vascular smooth muscles. Additionally, Cipriano et al found that ERGO maleate produced a similar degree of coronary arterial narrowing in the denervated heart transplantation patients to that of normally innervated hearts. These results, together with work using Langendorff preparations and isolated aortic strips, suggest that the mechanism of vasoconstriction caused by ERGO is a direct smooth muscle effect. Thus, the abnormalities in vasomotor reactivity occurring at the angioplasty site probably reflect alterations in the media rather than the effects of endothelium-dependent modulators of vascular tone.

**Limitations of This Model**

No model of human disease is perfect. The primary advantage of this model is that reproducible atherosclerotic lesions suitable for angioplasty can be produced relatively quickly and that the mechanism of successful dilatation as assessed histologically is similar to the limited available human data. The chief disadvantage is that the experimental lesion has a preponderance of foam cells, which are not seen in human disease, where the lesions tend to exhibit necrosis, fibrosis and calcification. In addition, the cholesterol levels in the experimental model are much higher than those seen in humans. Despite these limitations, this model provides information not readily obtainable from patients and does provide insight into the process of human angioplasty.

**Implications**

At the angioplasty site, vascular reactivity to the two agents tested was acutely abolished for all four protocols. This finding would imply that acute closure originating at the angioplasty site is not commonly caused by spasm but is probably due to a thrombus and/or dissection. However, it is important to recognize that the medial lesion produced in this model is concentric. Conceivably, dilatation of an eccentric lesion could protect a portion of the media from damage, thus allowing spasm to occur. Spontaneous spasm was prominent distal to the angioplasty site. In addition, vascular reactivity was preserved in all distal as well as in those proximal segments dilated using an appropriately sized balloon. Thus, in situations where the occlusion originates proximal or distal to the angioplasty site, spasm may be a contributing factor. There was no significant connection between the reactivity of the angioplasty segment to ERGO and NTG and later restenosis. The restenosis rate, defined as a 50% loss of the initial gain for the oversized and the appropriately sized balloon groups, were not significantly different [10 of 17 (59%) and five of nine (56%)]. However, we recognize that, though compelling, these data are derived from a small number of observations.

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