Determinants of Smooth Muscle Injury During Balloon Angioplasty

Tim A. Fischell, MD, Gordon Grant, BS, and Danna E. Johnson, MD

To study the determinants of smooth muscle injury during balloon angioplasty, we conducted a series of experiments to examine the effects of degree of arterial stretching, duration of balloon inflation, and arterial precontraction on smooth muscle injury after balloon angioplasty in isolated, perfused whole-vessel segments of rabbit aortas and dog carotid arteries. Freshly dissected rabbit thoracic aortas and dog carotid arteries were mounted in a muscle bath-perfusion chamber and perfused at 80 mm Hg. The proximal half of each aorta was dilated with a 5-mm (41±6% stretch), 6-mm (64±6% stretch), or 8-mm (97±9% stretch) balloon angioplasty catheter, and the uninjured half of each vessel served as the control. The vasoconstrictor behavior of the dilated segment was then assessed by dose-response testing; long-axis, ultrasonic imaging combined with computerized edge-detection image processing was used to measure changes in segmental internal vessel diameters that were induced by phenylephrine. A similar series of experiments was performed in dog carotid arteries with 5-mm balloon catheters (42±2% stretch) to compare the susceptibility to smooth muscle injury between elastic (aortic) and muscular (carotid) arteries. Additional experiments were performed to determine the roles of prolonged (30 minutes) balloon inflation and arterial precontraction on smooth muscle injury after balloon angioplasty. In rabbit aortas, the dilated arterial segments demonstrated normal reactivity to phenylephrine after dilatation with 5- and 6-mm balloons (p = NS versus control). Severe smooth muscle injury (histopathologically) with “arterial paralysis” was observed after severe stretch (8-mm balloon) and after 5-mm balloon dilatation (46±5% stretch) in precontracted vessels. Prolonged balloon inflations did not impair aortic vasoconstrictor behavior. Dog carotid (muscular) arteries demonstrated angioplasty-induced smooth muscle injury with less severe degrees of stretch (47–52% stretch). Geometric modeling suggests that medial stretching during balloon angioplasty of diseased vessels in vivo is in the range of 15–41%. We conclude that 1) relatively severe arterial stretching is required to injure smooth muscle when balloon angioplasty is performed in relaxed arteries, 2) prolonged balloon inflation does not alter the severity of smooth muscle injury after balloon dilatation in isolated arteries, 3) muscular arteries appear more susceptible to stretch-induced smooth muscle injury than do elastic arteries, 4) precontraction serves as a catalyst in promoting stretch-induced smooth muscle cell lysis and arterial paralysis, and 5) these findings are consistent with the clinical observations that coronary angioplasty rarely results in arterial paralysis. (Circulation 1990;82:2170–2184)

Balloon angioplasty is a widely accepted modality for the treatment of obstructive peripheral and coronary artery disease. Despite the widespread application of this therapy, there is still uncertainty regarding the mechanism(s) of successful angioplasty and the effects of balloon angioplasty on smooth muscle integrity and arterial vasoconstrictor behavior.1–8 Several clinical studies, including the reports from the original National Heart, Lung, and Blood Institute Registry, have described clinically obvious coronary artery spasm at the site of balloon dilatation despite premedication to prevent this complication.9–14 In contrast, animal studies in normal rabbit aortas,9,10 normal and diseased rabbit iliac arteries,15–21 and normal dog carotid arteries22 have suggested that balloon angioplasty reproducibly caused severe injury to the medial smooth muscle, resulting in “arterial paralysis” and rendering any dilated vessel incapable of vasocostriction. These observations in animals were the basis for a widely accepted theory that the principal mechanism of successful percutaneous...
transluminal coronary angioplasty (PTCA) was immediate and irreversible arterial “stretching” with aneurysm formation secondary to severe smooth muscle injury. The possibility that this arterial paralysis was the result of balloon oversizing or enhanced susceptibility to medial injury in these animal models was suggested by discrepancies between the severe medial injury observed histopathologically in the animal arteries and the milder injury observed in several human postmortem studies. The purpose of this study was to elaborate the determinants of smooth muscle injury caused by balloon angioplasty in perfused whole vessels under near-physiological conditions. Specifically, we addressed the following questions. 1) In relaxed arteries, what is the relation between the degree of balloon-induced arterial stretching and resultant smooth muscle injury? 2) Does prolonged balloon inflation influence the severity of angioplasty-induced smooth muscle injury? 3) Does arterial (smooth muscle) precontraction alter the susceptibility to stretch-induced smooth muscle injury? 4) Are there differences in susceptibility to stretch-induced smooth muscle injury between elastic (rabbit aorta) and muscular (dog carotid) arteries? 5) What is the expected or usual range of medial “stretching” during percutaneous transluminal coronary angioplasty in humans? These questions have become increasingly important in light of recent evidence suggesting that the balloon sizing and the degree of medial (smooth muscle) injury during balloon angioplasty may influence both the short- and long-term efficacy of the procedure.

Methods

An ultrasonic imaging technique was used to study the segmental vasomotor effects of balloon angioplasty in isolated, perfused rabbit thoracic aortas and dog carotid arteries. This technique uses high-frequency, long-axis ultrasonic imaging of vessels suspended and perfused in a muscle bath and uses computerized edge-detection image processing to measure segmental internal vessel diameters. Dose-response curves to pharmacological agents obtained by this method have shown a high correlation with data obtained by classic tissue bath techniques with arterial ring segments and force displacement transducers. The experimental apparatus used for these studies is shown in Figure 1.

Tissue Collection and Preparation

The thoracic aortas from 38 New Zealand White rabbits (females, 1–3 kg) and nine dog common carotid arteries were used for the series of experiments described below. All studies were performed in compliance with the Stanford University Panel on Animal Laboratory Care guidelines. The rabbits were killed by cerebral concussion, and their thoracic aortas, approximately 4 cm in length, were dissected free. The dogs were anesthetized with intravenous morphine sulfate and pentobarbital sodium general anesthesia before being killed. The common carotid arteries were dissected free, transected at their origin and at the carotid bifurcation, and removed. The arteries were mounted in the muscle bath by tying the proximal and distal ends of the vessel to the tapered and adjustable vessel attachment pieces with 2-0 silk suture as shown in Figure 1. The arteries were suspended to approximate their original in vivo length. The vessels were bathed in physiological saline solution with the following composition (mM): 24 NaCl, 4 KC1, 1.2 MgSO4, 2 CaCl2, 5 dextrose, 24 NaHCO3, and 1.2 Na2HPO4. The pH was kept constant at 7.40–7.45, and the muscle bath solution was warmed to 36–37°C and aerated with 95% O2-5% CO2. All perfusates were heated to 36–37°C by passage through a capillary tubing network within the outer heating bath and then delivered to the proximal vertical port by standard polyvinyl chloride intravenous tubing.

To permit perfusion at physiological pressures and flow rates without side branch leakage, all of the small intercostal arteries arising from the posterior aspect of the rabbit aortas were cauterized using a miniature, battery-powered surgical cautery device (Accu-Temp, Concept, Inc., Clearwater, Fla.). The cautery was performed at a minimum distance of 0.5 mm from the lumbar artery takeoff to ensure that there would be no thermal damage to the thoracic aorta. The dog common carotid artery lacks side branches and, therefore, did not require cautery before study.

Balloon Angioplasty Studies

After mounting, the arteries were perfused with recirculating oxygenated physiological saline solution, with a flow rate maintained at 50–60 ml/min by a constant flow (nonpulsatile) peristaltic pump. Vessel perfusion pressure was maintained at a constant 80 mm Hg throughout each study by minor adjustments in outflow resistance. Minor vessel length adjustments were made to ensure that there was no resting longitudinal tension at this perfusion pressure. The vessels were allowed to equilibrate for 45 minutes before experimental manipulation. After equilibration perfusion, a 10-mHz ultrasonic transducer (200 RF, Diasonics, Milpitas, Calif.) was positioned approximately 1 cm above the artery in contact with the muscle bath solution. The transducer head was aligned along the vessel to optimize the long-axis image of the upper and lower vessel walls (Figure 1). Gain settings were held constant throughout the experiment. This system provided a uniform, sharp vessel wall image of at least 3 cm of vessel length at short focal distances (less than 1 cm). Once the vessel was optimally imaged, real-time ultrasonic vessel wall images were recorded to determine the control vessel internal diameter. All ultrasonic images were recorded with a ¾-in. videocassette tape recorder (Sony U-Matic, VO-5800).

Balloon angioplasty was then performed in the proximal vessel segment relative to perfusate flow; the balloon catheter was introduced by the proximal central port. In all experiments other than the prolonged balloon inflation experiments (see below),
three 60-second inflations to 6 atm, each separated by 15 seconds, were performed with catheters of varying inflated balloon diameters (see below) with a balloon length of 2.0 cm. The inflated balloon diameters for each set of experiments were chosen as described below.

Effect of Balloon Size (Stretch)

To determine the effects of severity of arterial stretching on smooth muscle integrity, a series of experiments was performed with angioplasty catheters with inflated balloon diameters of 5 mm ($n=8$), 6 mm ($n=8$), or 8 mm ($n=8$) in rabbit aortas. These balloon catheters were chosen to provide increasing degrees of arterial stretching, with percent stretch defined as follows: \[
\text{% stretch} = \left(\frac{\text{balloon size [mm]} - \text{relaxed internal vessel diameter [mm]}}{\text{relaxed internal vessel diameter [mm]}}\right) \times 100.
\]

After the balloon dilatations, as described above, the vessels were perfused with physiological saline perfusate containing increasing log doses of phenylephrine ($10^{-8}$ to $10^{-4}$ M). Real-time ultrasonic vessel images were observed continuously during and after angioplasty, and vessel images were recorded during balloon inflations, immediately after balloon catheter removal, and after equilibration vasoconstriction (i.e., no further change in diameter observed during a 10-minute period) during dose-response testing with phenylephrine. Light and transmission electron microscopic examinations of control and balloon angioplasty segments were performed as described below.

Effect of Prolonged Balloon Inflation

Another series of experiments was performed to compare the effects of “short” (as above) with prolonged balloon inflation on smooth muscle function.
Thus, phenylephrine dose-response testing was performed in six rabbit aortas after prolonged balloon inflation (6-mm balloon, one 30-minute balloon inflation), and these results were compared with those obtained by a more conventional inflation protocol (6-mm balloon, three 1-minute inflations).

Effect of Smooth Muscle Precontraction

It was incidentally noted during one of the preliminary experiments that even modest angioplasty-induced stretching impaired vasoconstrictor responses in rabbit aortas that were precontracted with either serotonin or phenylephrine. To evaluate the effects of arterial (smooth muscle) contractile state on susceptibility to stretch-induced smooth muscle injury, we evaluated the vasoconstrictor responsiveness to high-dose phenylephrine ($10^{-4}$ M, 2 hours of observation) in rabbit aortas dilated with 5-mm balloons ($n=8$) after precontraction with $10^{-3}$ M phenylephrine. These data were compared with those obtained from rabbit aortas dilated with the same size (5-mm) balloon catheters but without precontraction. Light and transmission electron microscopic examination of control and balloon angioplasty segments were performed as described below.

Muscular and Elastic Arteries

To assess the differences in susceptibility to stretch-induced injury, if any, between elastic (rabbit aortas) and muscular arteries, another set of experiments was performed to determine the effects of balloon angioplasty on smooth muscle function in dog carotid (muscular) arteries ($n=9$). In these experiments, dog carotid arteries with resting diameters ($3.61 \pm 0.10$ mm) similar to those of rabbit thoracic aortas ($3.59 \pm 0.15$ mm) were dilated with 5-mm balloon catheters. Dose-response testing with increasing concentrations of phenylephrine was performed (as above). Light microscopic examinations of control and balloon angioplasty segments were performed in the dog carotid arteries as described below.

Data Collection and Computerized Edge Detection

Mean internal vessel diameters of the control (non-manipulated) and balloon angioplasty segments, measured throughout a 1-cm length, were determined by computerized edge-detection image processing of the two-dimensional ultrasonic images. The application of this computerized edge-detection system to analysis of internal vessel diameters from long-axis ultrasonic vessel wall images has been previously described in detail.29,30 The overall resolution of the imaging and image-processing system has been calculated to be $\pm 0.22$ mm, using a 10-mHz transducer.

Histologic Examination

At the conclusion of the experiments, one ring segment, approximately 2 mm in length, was cut from both the control and the balloon angioplasty segments. The ring segments were fixed in 1% glutaraldehyde in 0.067 M cacodylate buffer. Ring segments were examined by light microscopy (hematoxylin and eosin staining) by a vascular pathologist in a blinded fashion for each of the following subgroups: 1) rabbit aorta, control ($n=10$); 2) rabbit aorta, 5-mm balloon without precontraction ($n=5$); 3) rabbit aorta, 6-mm balloon without precontraction ($n=5$); 4) rabbit aorta, 8-mm balloon without precontraction ($n=5$); 5) rabbit aorta, 5-mm balloon after precontraction ($n=5$); and 6) rabbit aorta, 6-mm balloon with 30-minute inflation ($n=5$). Ring segments from all nine dog carotid artery experiments were performed in a blinded fashion by light microscopy; accordingly, each segment was qualitatively graded for the presence (>50% intact) or absence of endothelium, presence or absence of pyknotic smooth muscle cell nuclei, and the presence or absence of sign(s) of smooth muscle cell lysis or necrosis. Transmission electron microscopy ($\times 1,000$–$28,000$) was performed in an unblinded fashion for four ring segments in each of the six subgroups above; each ring segment was qualitatively graded for the presence or absence of cell membrane disruption, pyknotic nuclei, mitochondrial edema, swelling of endoplasmic reticulum, and disruption of dense bodies.

Statistical Analysis

Student’s $t$ test for paired means was used to compare the mean vessel diameters of the control and balloon angioplasty segments before and after angioplasty and the contraction with high-dose phenylephrine in the precontraction experiments. A $t$ test was used to compare the slopes of the linear regression lines describing the effects of stretch on impairment of vasoconstriction in rabbit aortas and in dog carotid arteries. Analysis of variance (repeated-measures, Fisher’s LSD) was used to compare the phenylephrine dose-response curves of control and dilated vessel segments in rabbit aortas after dilatation with 5-, 6-, and 8-mm balloons, the effects of prolonged in comparison to short balloon inflation, and the differences in vasoconstrictor responsiveness after balloon dilatation between elastic and muscular arteries. Unless otherwise stated, data are presented as the mean $\pm$ SEM.

Drugs

$1\text{-Phenylephrine hydrochloride was obtained from Sigma Chemical Co., St. Louis, Mo. All drugs were prepared fresh on the day of the experiments. Phenylephrine was dissolved initially in distilled water and then added in precalculated volumes (0.4 ml) to physiological saline to achieve the desired drug concentration in each perfusate.}$

Results

Influence of Stretch After Balloon Angioplasty

In the 5-mm balloon experiments ($n=8$), the rabbit aortas had a control diameter of $3.59 \pm 0.15$ mm and were stretched by an average of 41$\pm 6$%
beyond their relaxed internal vessel diameters. In the 6-mm balloon experiments (n=8), the aortas (control diameter, 3.69±0.18 mm) were stretched by 64±6% beyond their relaxed diameters, and in the 8-mm balloon experiments (n=8), the aortas (control diameter, 4.02±0.43 mm) were stretched by 97±9% beyond their resting diameters.

Dose-response testing with phenylephrine demonstrated normal vasoconstrictor responses in the balloon angioplasty segment after dilatation with 5- and 6-mm balloons (Figure 2A and B). There were no significant differences in the response to phenylephrine at any dose (10^-8 to 10^-4 M) between the balloon angioplasty and control (nondilated) segments.

In contrast, there was marked impairment of vasoconstrictor responsiveness after the severe stretching (8-mm balloon dilatation) with nearly complete arterial paralysis (Figure 2C). The differences in response to phenylephrine between the control segments and the injured segments were statistically significant (p<0.01) at phenylephrine doses of 10^-7, 10^-6, 10^-5, and 10^-4 M. The light microscopy and transmission electron microscopy correlated well with the vasomotor data (Tables 1 and 2). The aortas dilated with 5- or 6-mm balloons demonstrated endothelial denudation without signs of significant smooth muscle injury, whereas the segments dilated with the 8-mm balloons generally showed signs of severe smooth muscle cell injury.
with smooth muscle cell lysis (Figures 3A–C and 4A–C, Tables 1 and 2).

**Effects of Prolonged Balloon Inflation**

Six rabbit aortas (control diameter, 3.55±0.18 mm) were dilated for 30 minutes each with 6-mm balloons (69±5% stretch). There was no significant difference in the degree of stretching between short and long balloon inflations of the aortas dilated with 6-mm balloons. The aortas treated with long inflations demonstrated normal (i.e., same as control segment) vasoconstriction to phenylephrine. The vasoconstrictor responses did not differ from those after short (see above) 6-mm balloon inflations (Figure 2D).

**Effect of Precontraction on Susceptibility**

Eight rabbit aortas (control diameter, 3.48±0.19 mm) were dilated with a 5-mm balloon (46±5% stretch beyond relaxed diameter) after precontraction with 10⁻³ phenylephrine. The average vessel precontraction (in percent, compared with resting diameter) at this dose was 37±3%, yielding a precontracted vessel diameter of 2.19±0.15 mm. The actual stretching imparted by the 5-mm balloon beyond the precontracted vessel diameter was 128±8%.

Balloon angioplasty (5-mm balloon) after precontraction resulted in a nearly complete loss of vasoconstrictor responsiveness in all aortas, which persisted for at least 2 hours. Typical ultrasonic images demonstrating the severe impairment of vasoconstrictor responsiveness in the dilated segment is shown in Figure 5. The impairment of vasoconstrictor responsiveness was significantly different from the normal vasoactivity observed in similarly sized rabbit aortas dilated with 5-mm balloons without precontraction (Figure 6). Histopathological examination of these aortas dilated with a 5-mm balloon after precontraction revealed severe smooth muscle injury and cell lysis (Figures 3D and 4D). Transmission electron microscopy of aortas dilated with 5-mm balloons after precontraction demonstrate potentially reversible signs of cell injury such as cellular edema, swelling of mitochondria and rough endoplasmic reticulum, and diffuse signs of irreversible injury that included chromatin clumping, pyknotic nuclei, cell membrane disruption, and dispersed cellular organelles (Figure 4D). These signs of smooth muscle cell injury in precontracted vessels appeared to be more extensive than those observed after severe stretching with 8-mm balloons in relaxed aortas (Figure 4C).

**Stretch-Induced Injury in Muscular and Elastic Arteries**

Nine dog carotid arteries (control diameter, 3.61±0.10 mm) were dilated with a 5-mm balloon catheter, yielding an average stretch of 42±2% beyond the relaxed vessel diameter. Dose-response testing to phenylephrine after balloon dilatation demonstrated moderate impairment of vasoconstriction (maximal constriction, 25–75% of control) in three vessels and severe impairment (maximal constriction, 2% of control) in one vessel. The other

---

**Table 1. Light Microscopy Results**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n</th>
<th>Endothelial cell disruption (&gt;50%)</th>
<th>Smooth muscle cell necrosis</th>
<th>Pyknotic nuclei (smooth muscle)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>1/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>5-mm balloon</td>
<td>5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>6-mm balloon</td>
<td>5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>8-mm balloon</td>
<td>5</td>
<td>5/5</td>
<td>4/5</td>
<td>5/5</td>
</tr>
<tr>
<td>5-mm balloon (precontracted)</td>
<td>5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>6-mm balloon (long inflation)</td>
<td>5</td>
<td>5/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*All four vessels with smooth muscle cell necrosis demonstrated significant (>25%) impairment of vasoconstrictor responsiveness (see Figure 7).

---

**Table 2. Transmission Electron Microscopy Results (Smooth Muscle)**

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>n</th>
<th>Cell membrane disruption</th>
<th>Chromatin clumping</th>
<th>Mitochondrial edema</th>
<th>Endoplasmic reticulum edema</th>
<th>Disrupted dense bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit aorta</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>5-mm balloon</td>
<td>4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>6-mm balloon</td>
<td>4</td>
<td>0/4</td>
<td>1/4</td>
<td>0/4</td>
<td>1/4</td>
<td>0/4</td>
</tr>
<tr>
<td>8-mm balloon</td>
<td>4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>5-mm balloon (precontracted)</td>
<td>4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>3/4</td>
</tr>
<tr>
<td>6-mm balloon (long inflation)</td>
<td>4</td>
<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
<td>1/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>
dilated segments demonstrated vasoconstrictor responsiveness that was either equal to or greater than their respective control segments (Figure 7). Impaired smooth muscle vasoreactivity was associated with a greater degree of stretch. The average stretch in the four carotid arteries showing impaired vasoconstriction was 49±3% compared with 33±3% for the unimpaired segments (p<0.01, ANOVA). In contrast to the findings in relaxed rabbit aortas (elastic arteries), which were uninjured after stretch of up to 76%, the muscular (carotid) arteries showed signs of smooth muscle injury with more moderate stretching (i.e., 47–52%). Even so, practically no smooth muscle injury was observed in muscular arteries stretched by 45% or less beyond their relaxed internal vessel diameter. A comparison of the effects of varying degrees of stretch in dog carotid arteries compared with rabbit aortas is shown in Figure 7. Figure 8B is a photomicrograph showing smooth muscle cell lysis (arrows) in a dog carotid artery stretched by 51% beyond its relaxed internal vessel diameter.

**Discussion**

Prior studies of balloon angioplasty in animal models suggest that the medial stretching imparted by balloon angioplasty routinely caused severe smooth muscle injury, acutely disabling the arterial contractile apparatus, resulting in arterial paralysis. These observations in normal and diseased rabbit iliac arteries and normal dog and pig carotid arteries provided the basis for a widely accepted theory that the principal mechanism of successful PTCA was immediate and irreversible arterial stretching and that aneurysm formation was secondary to severe smooth muscle injury. In contrast to these observations in animal tissue, coronary vasoconstriction or frank spasm has been observed in the dilated coronary segment early and late after successful balloon angioplasty in humans. The results from this study, in which the detailed histologic and vasomotor consequences of varying degrees and types of balloon-induced arterial stretching were examined in a perfused whole-vessel ex vivo model, help to better delineate the determinants of acute smooth muscle injury during balloon angioplasty. These findings may have important implications for determining the cause, and prescribing treatment, of coronary vasoconstriction, abrupt vessel closure, and possibly restenosis after PTCA.
FIGURE 4. Transmission electron microscopic examination of the varying effects of balloon angioplasty in rabbit aorta (original magnification, ×4,000). Panel A: Uninjured (control) segment with normal-appearing smooth muscle cells (Sm, smooth muscle cell; Nu, cell nucleus). Panel B: Segment dilated with a 5-mm balloon without loss of vasoconstrictor responsiveness also exhibits nearly normal-appearing smooth muscle cell architecture (see text). Smooth muscle cell injury with cellular edema, cystically dilated rough endoplasmic reticulum (rer), swollen mitochondria (mc), and disruption of the cell membrane (arrows) is seen in a "paralyzed" arterial segment after severe stretching by an 8-mm balloon catheter (panel C). A similar but more severe pattern of smooth muscle cell injury and lysis, with pyknotic cell nuclei (pNu) and dispersion of cellular organelles is apparent after dilatation with a 5-mm balloon in a precontracted arterial segment (panel D).
Relation of Severity of Stretch to Smooth Muscle Injury

In rabbit aortas and dog carotid arteries, a clear-cut relation exists between the degree of arterial stretching beyond the relaxed internal vessel diameter and the severity of smooth muscle injury as determined by loss of vasoconstrictor responsiveness and by histopathological examination.

In the elastic rabbit aorta, there appears to be a threshold effect of stretch-induced injury such that there is minimal or no injury with less than 80% stretch but severe and reproducible injury with stretching of more than 90–95%. These findings correlate well with the observations made by Wolf et al. that impaired vasoconstrictor responsiveness, in vitro, occurs when rabbit aortas are stretched to greater than 90% beyond their resting diameters. The mechanism of smooth muscle injury and loss of vasoconstrictor reactivity after severe stretching in relaxed rabbit aortas appears to be smooth muscle cell lysis, possibly due to overstretching of the cell membrane beyond its physical limits with resultant tearing and loss of membrane integrity. Light microscopy suggests that the more superficial layers of media, which undergo proportionately greater stretching, are more vulnerable to stretch-induced injury than are the deeper medial layers. Transmission electron microscopy confirms that there are minimal signs of reversible cell injury or no structural changes in rabbit aortas stretched by 40–80%. These findings contrast with the diffuse areas of cell lysis and necrosis observed after severe stretching (i.e., >90%). The histologic pattern of smooth muscle cell lysis was only observed in those aortas that also demonstrated significant loss of vasoconstrictor responsiveness (Tables 1 and 2). We were unable to demonstrate any significant effect of prolonged balloon inflation on smooth muscle contractility or viability in rabbit aortas.

Unlike the compliant, elastic rabbit aortas that teleologically should be adapted to tolerate stretch, the muscular dog carotid artery and other muscular arteries, including human coronary arteries (unpublished data), appear to be somewhat more susceptible to stretch-induced smooth muscle cell injury. Whereas rabbit aortas require 105% stretch beyond

![Figure 5. Long-axis ultrasonic images demonstrating “arterial paralysis” after balloon angioplasty after precontraction in rabbit aorta. Panel A: Long-axis ultrasonic image of rabbit aorta after equilibration perfusion with physiological saline solution (diameter, 3.5 mm). Panel B: Vessel was precontracted by perfusion with phenylephrine 10^{-5} M (precontracted diameter, 2.3 mm). After balloon angioplasty of the proximal half of the vessel (5-mm balloon, 43% stretch beyond relaxed vessel diameter), there is loss of vasoconstrictor responsiveness in the injured segment (diameter, 3.7 mm) with unchanged vasoconstriction to phenylephrine 10^{-5} M in the distal (control) segment (diameter, 2.3 mm) (panel C).](http://circ.ahajournals.org/content/82/6/2178)
their relaxed diameter to induce a 50% loss of vasoconstrictor responsiveness, dog carotid arteries suffered the same (50%) impairment of vasoconstrictor responsiveness with stretching of 51% beyond their relaxed diameter, a significant difference (Figure 7). Similarly, in a postmortem study of balloon angioplasty, Saffitz and colleagues found that equivalent dilatory force created greater damage in the distal (muscular) than in the proximal (elastic) portion of human renal arteries. The increased vulnerability to stretch-induced injury in muscular arteries is probably best explained by the decreased compliance of muscular arteries compared with elastic arteries. In addition, the smooth muscle cells in muscular arteries may be more closely attached to each other or the extracellular matrix by fibronectin-dependent attachments to collagen types I and III. The shearing forces applied to the cell membranes by these intercellular connections during rapid and severe stretching may disrupt the smooth muscle cell membranes, resulting in cell lysis and impaired vasoconstrictor responsiveness. Despite this relative sensitivity to stretch-induced injury in muscular arteries compared with elastic arteries, moderately severe arterial stretching is still required to impair smooth muscle vasoconstriction in muscular arteries during balloon angioplasty.

**Arterial Precontraction as a Catalyst for Smooth Muscle Injury During Balloon Angioplasty**

One of the more intriguing findings of these studies was the relation between smooth muscle contraction and the predisposition to balloon-induced (stretch) smooth muscle injury. In precontracted rabbit aortas, balloon angioplasty with modestly sized balloons that would be expected to cause minimal or no smooth muscle injury in relaxed vessels resulted in extensive smooth muscle cell lysis and arterial paralysis. Although the explanation for this phenomenon is not certain, it is likely that the activation of the actin-myosin contractile apparatus, which anchors into the cell membrane by the dense bodies, increases the smooth muscle cell stiffness in precontracted vessels. In *Dictyostelium* cells, for example, which have an intracellular arrangement of actin and myosin filaments similar to vascular smooth muscle cells, activation of actin-myosin cross-bridges markedly increases mechanical measures of cellular stiffness. This contraction-induced "stiffness" with an associated increase in the tension of the actin filaments anchored into the cell membrane may predispose the smooth muscle cell to membrane lysis when it is acutely distended during balloon angioplasty.

This finding that precontraction predisposes arteries to stretch-induced smooth muscle injury may help to explain some of the prior observations regarding arterial behavior after balloon angioplasty. Although speculative, the effectiveness of repeated balloon dilatation in the treatment of abrupt vessel closure...
Figure 8. Light microscopic examination of the effect of balloon angioplasty in dog carotid artery. Panel A: Uninjured (control) segment with an intact endothelial monolayer on the luminal surface (L) and normal-appearing smooth muscle cells. Panel B: Smooth muscle cell loss (arrows), pyknotic smooth muscle cell nuclei, and endothelial denudation are seen in an arterial segment after stretching (51% beyond relaxed diameter) by a 5-mm balloon catheter. Hematoxylin and eosin staining; original magnification, ×450.

After PTCA (73–95% effective in multiple reports) may be related, in part, to this phenomenon. For example, in certain cases of abrupt closure, the initial balloon inflation in a coronary artery with little or no initial tone may provoke arterial vasoconstriction that then predisposes the vessel to arterial paralysis with subsequent balloon inflations, yielding an excellent final result. Similarly, the efficacy of laser thermal balloon angioplasty in treating abrupt closure may be related to acute and severe smooth muscle cell (thermal) injury preventing vasoconstriction or elastic recoil.

This phenomenon of vasoconstriction predisposing to stretch-induced smooth muscle injury may also explain the frequent finding of arterial paralysis after balloon angioplasty in spasm-prone rabbit arteries in vivo. This hypothesis is supported by the observation that arterial spasm (presumably
FIGURE 9. Schematic of the severity of medial stretching during balloon angioplasty in diseased human coronary arteries. Panels A, B, and C: "Least stretch" scenario in which there is intimal thickening in the "normal" reference segment, compensatory medial hypertrophy in the dilated segment, and a 50% (angiographic) luminal narrowing, yielding 16% medial stretching with balloon dilatation (assuming the plaque is incompressible). Panels D, E, and F: "Greatest stretch" or worst-case scenario with no intimal thickening in the reference segment, no medial hypertrophy in the dilated segment, and a total occlusion, yielding a 41% stretch with balloon inflation.

catheter induced) was angiographically evident in the rabbit aortas at the time of balloon dilatation in the study by Wolf et al.\textsuperscript{8}

Clinical Implications

To extrapolate the findings from these studies to the clinical setting, it is important to try to estimate the severity of medial stretching caused by balloon angioplasty in diseased arteries in vivo. If one uses a geometric model of the coronary artery before and during balloon angioplasty, with the assumptions that the plaque is incompressible,\textsuperscript{42} is not extruded along the vessel,\textsuperscript{42} does not embolize, and that the balloon is fully inflated, one can estimate the range of medial stretching during PTCA in humans. Admittedly, this model represents a simplification of the pathological processes that occur during angioplasty, but if anything, it overestimates the degree of medial stretching. That is, if there were plaque compression, longitudinal extrusion, plaque embolization, or incomplete balloon inflation, the actual stretching of the media would be less than that calculated below.

The purpose of this analysis is to emphasize how relatively little arterial stretching may occur in vivo.

In Figure 9, two sets of diagrams are shown illustrating a "least (or little) stretch" (panel A) and a "greatest stretch" (panel B) scenario during PTCA in humans. In both scenarios, it is assumed that the balloon size is chosen to match the diameter of an angiographically normal arterial segment adjacent to the lesion to be dilated.\textsuperscript{26} The calculation of the percent stretch is based on the change in diameter of the internal elastic lamina before and during balloon inflation, which is the same definition that was used in the in vitro experiments. The deeper medial layers will be stretched less than the internal elastic lamina. Panel A (least stretch) depicts the more likely scenario in which the angiographic diameter of the "normal" segment underestimates the true medial diameter due to unappreciated diffuse intimal thickening.\textsuperscript{43} In addition, there is modest medial hypertrophy of the diseased (dilated) segment as a compensatory response to atherosclerosis.\textsuperscript{44,45} As a result, a 3.0-mm balloon is chosen to dilate a 50%
stenosis in a vessel with a 4.5-mm internal elastic lamina (medial) diameter, yielding 16% or less medial stretching beyond the relaxed diameter. Based on the data presented for both rabbit aortas and dog carotid arteries, one would predict that this minor degree of medial stretching would cause minimal smooth muscle injury and would have no significant effect on smooth muscle vasoconstrictor responsiveness. In panel B, a worst-case (greatest stretch) scenario is shown in which there is no intimal thickening in the reference segment adjacent to a total occlusion and no medial hypertrophy in the severely diseased (dilated) segment. In this relatively unlikely worst-case scenario, there is still only a modest 41% or less stretch of the media beyond its relaxed diameter. Minimal smooth muscle injury with little impairment of vasoconstrictor responsiveness would be expected to occur with this severity of arterial stretching in relaxed muscular (e.g., coronary) arteries. As emphasized above, the actual severity of stretching would be less than that calculated if there were any degree of plaque compressibility or plaque extrusion or if the balloon were modestly undersized (inflated balloon diameter to vessel diameter ratio, <1.0), as is often the case in clinical practice.

Despite the limitations of this type of analysis (e.g., may not adequately describe the arterial trauma during balloon angioplasty in highly eccentric lesions or with arterial laceration), it does serve to illustrate how modest the circumferential medial stretching is during PTCA. PTCA in humans likely results most often in medial stretching of only 20–30% beyond the relaxed coronary artery diameter. If this is true, based on the ex vivo data presented, one would not expect to see arterial paralysis after PTCA in humans unless one intentionally oversized the balloon catheter or if the artery were in a precontracted state before balloon inflation. The clinical observation of spontaneous coronary artery vasoconstriction or occasionally frank arterial spasm in the dilated segment after PTCA is consistent with this analysis.

Although the exact relation between the severity of medial smooth muscle injury and restenosis has not been fully defined, there is increasing evidence that reversible or irreversible smooth muscle injury during balloon angioplasty or atherectomy may provide a stimulus for smooth muscle cell proliferation, leading to intimal hyperplasia and restenosis. If stretch-induced smooth muscle injury were a significant predisposing factor in restenosis, one would predict based on the data presented that oversizing balloons and performing PTCA in vessels with increased arterial tone or spasm would lead to greater smooth muscle injury and higher restenosis rates. Although balloon oversizing has not been clearly shown to influence restenosis rates after PTCA, balloon oversizing and higher balloon inflation pressures leading to greater smooth muscle injury in a more controlled animal model have been shown to enhance intimal hyperplasia. Higher restenosis rates after balloon angioplasty have been observed in patients with variant angina and after repeated balloon angioplasty in the setting of abrupt closure. The increased incidence of restenosis in these patient groups may be due to coronary artery vasoconstriction at the time of angioplasty, leading to more severe stretch-induced smooth muscle injury, as discussed above, ultimately causing greater restenosis.

Summary

A series of experiments was performed in elastic (rabbit aortas) and muscular (dog carotid) arteries to delineate the determinants of stretch-induced smooth muscle injury during balloon angioplasty. These studies demonstrate that moderately severe arterial stretching is required to significantly impair smooth muscle vasoconstrictor responses when balloon angioplasty is performed in relaxed arteries. Prolonged balloon inflation does not alter the severity of smooth muscle injury after balloon dilatation in isolated arteries. Muscular arteries are more susceptible to stretch-induced smooth muscle injury than are elastic arteries but still require stretching of more than 50% beyond their relaxed diameter to cause arterial paralysis. Arterial vasoconstriction serves as a catalyst in promoting stretch-induced smooth muscle cell lysis and arterial paralysis, probably as a result of increased smooth muscle cell stiffness predisposing to tearing of the cell membrane during rapid stretching. Geometric modeling suggests that medial stretching during balloon angioplasty of diseased vessels in vivo is typically modest (range, 15–41%) and, therefore, not sufficient to cause severe smooth muscle injury or loss of vasoconstrictor responsiveness. These findings are consistent with the clinical observations that percutaneous transluminal coronary angioplasty rarely results in arterial paralysis.

References

15. Waller BF, Gorfinikel HJ, Rogers FJ, Kent KM, Roberts WC: Early and late morphologic changes in major epicardial coronary arteries after percutaneous transluminal coronary angioplasty. Am J Cardiol 1984;53:42C-47C
37. Pasternak C, Spudich JA, Elson EL: Capping of surface receptors and concomitant cortical tension are generated by conventional myosin. Nature 1989;341:549-551
42. Chin AK, Kinney TB, Ruirk GW, Shoour PM, Fogarty TJ: A physical measurement of the mechanisms of transluminal angioplasty. Surgery 1984;95:196-201


**KEY WORDS**  
spasm • smooth muscle • phenylephrine • precontraction
Determinants of smooth muscle injury during balloon angioplasty.
T A Fischell, G Grant and D E Johnson

Circulation. 1990;82:2170-2184
doi: 10.1161/01.CIR.82.6.2170

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/82/6/2170