Laboratory Investigation

Effect of Chronic Subcutaneous or Intramural Administration of Heparin on Femoral Artery Restenosis After Balloon Angioplasty in Hypercholesterolemic Rabbits
A Quantitative Angiographic and Histopathological Study

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Background. Heparin is known to have antithrombotic, anticoagulant, and antiproliferative effects. We hypothesized that chronic subcutaneous and/or direct intramural administration of heparin would reduce restenosis and inhibit plaque growth after balloon angioplasty.

Methods and Results. Focal atherosclerosis was induced bilaterally in the femoral arteries of 59 rabbits by air desiccation intimal injury and a 2% cholesterol diet. After angioplasty, the rabbits were assigned to one of four treatment groups. Control arteries (n = 21) received no additional heparin. A second group of 20 arteries was treated with a porous balloon that delivered heparin (1,500 units) directly into the arterial wall. A third group (n = 29) received subcutaneous heparin (350 units·kg·day⁻¹) for 28 days, and a fourth group (n = 23) was treated with subcutaneous and intramural heparin. Quantitative angiography showed a modest reduction in restenosis (defined as the change in minimal luminal diameter from immediately after angioplasty to 28 days) with subcutaneous heparin compared with control arteries (0.32±0.18 versus 0.58±0.34 mm, p<0.01); however, luminal diameter was not improved at 28 days compared with before angioplasty. Intramuscular delivery of heparin by the porous balloon catheter was confirmed by use of fluorescein-labeled heparin in one animal. Angiographic restenosis was not reduced in arteries treated with intramuscular heparin versus controls (0.61±0.54 versus 0.58±0.34 mm, p=NS). Blinded planimetric analysis of histological sections showed no differences in luminal cross-sectional area narrowing by atherosclerotic plaque, in plaque area, or in plaque/media ratio at 28 days among the four treatment groups.

Conclusions. Chronic subcutaneous heparin after balloon angioplasty results in a modest reduction in angiographic restenosis in this model; however, the absolute luminal diameter is not improved compared with before angioplasty, and plaque area and percent luminal narrowing by plaque were not different among the four treatment groups. Heparin can be delivered into an atherosclerotic plaque by a porous balloon, but this treatment does not reduce restenosis after angioplasty in this model. (Circulation 1992;86:1536–1546)

KEY WORDS • restenosis • angioplasty • heparin • balloons

D espite its increasing use for the relief of atherosclerotic coronary artery disease, percutaneous transluminal coronary angioplasty remains plagued by a 25–35% frequency of restenosis.1 Numerous biological and mechanical strategies have been used in humans to reduce the rate of restenosis, but no therapy has consistently shown benefit.2–4 Restenosis is believed to result from a complex interaction of thrombosis, cellular proliferation, and elastic recoil.5–7 Thrombus formation associated with vascular injury may be important in initiating cellular growth.8 Heparin is known to have both anticoagulant and antiproliferative effects.9 Its anticoagulant action results from marked acceleration of antithrombin III–mediated neutralization of serine proteases, including thrombin.10 Smooth muscle cell proliferation in balloon-injured arteries is inhibited by heparin, and heparin has been shown to regulate entry of the smooth muscle cell into the S phase of its cycle.11 Smooth muscle cell migration

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and proliferation appear to be specifically inhibited by heparin, whereas endothelial regeneration is not. Both anticoagulant and nonanticoagulant heparin fragments have been developed that inhibit the proliferation of vascular smooth muscle cells in vivo and in vitro.

There is increasing interest in “site-specific” or “direct” delivery of antiproliferative agents into the arterial wall. Edelman and associates reported the inhibition of smooth muscle cell proliferation after vascular injury by surgical placement of a heparin-impregnated polymer matrix in the periadventitial tissues of rat carotid arteries. This route of administration was more effective than either intravenous or subcutaneous delivery of heparin. The recent development of a catheter-based porous balloon has permitted the application of “site-specificity” to percutaneous techniques. Wolinsky and associates have documented the feasibility of this concept using heparin in the normal canine artery and in postmortem specimens. The present angiographic and histopathological study investigates the effects of chronic subcutaneous (systemic) and/or intramural (site-specific) delivery of heparin on restenosis after angioplasty of atherosclerotic femoral arteries in rabbits.

Methods
The experimental design is summarized in Figure 1. Details of this experimental model have been published previously.

Induction of Focal Atherosclerosis
Fifty-nine male New Zealand White rabbits (weight, 4.0±0.4 kg) were anesthetized with ketamine (50 mg/kg i.m.) and xylocaine (5 mg/kg i.m.). Femoral artery segments (1–2 cm in length) were exposed bilaterally 1 cm below the inguinal ligament and secured between airtight ligatures. Endothelial damage was induced by air desiccation with nitrogen gas. Beginning the next day, animals were fed a diet consisting of 2% cholesterol and 6% peanut oil for 1 month. We have previously shown that cholesterol levels increase approximately 20- to 25-fold with this diet. After angioplasty, the rabbits received standard rabbit chow. Procedures were performed with sterile technique and general anesthesia, and studies conformed in all ways to the guidelines stated in the “Position of the American Heart Association on Research Animal Use.”

Angioplasty
Balloon angioplasty was performed 36±6 days (range, 27–39 days) after endothelial injury. After withdrawal of blood for baseline activated partial thromboplastin time, all rabbits received an intra-arterial bolus of heparin (150 units/kg, heparin sodium injection, porcine intestinal mucosa, 1,000 USP U/ml, Solutopak Laboratories, Franklin Park, Ill.) and xylocaine (20 mg). A baseline angiogram of the iliac and femoral arteries
was performed via the carotid artery using a 5F Berman angiographic balloon catheter (Arrow International, Inc., Reading, Pa.) positioned two vertebral spaces above the aortoiliac bifurcation and a Siemens Optilux Angiographic System (model 1179878VS048, Siemens AG, Munich). Images were recorded on 35-mm cineangiographic film. The angiographic catheter was replaced by a 0.014 guide wire, and a 2.5-mm-diameter ACS balloon dilation catheter (Advanced Cardiovascular Systems, Inc., Temecula, Calif.) was advanced across the femoral stenosis. Three 60-second, 10-atm balloon inflations were performed 1 minute apart with a hand inflator, and the in vivo balloon dimension was recorded by cineangiography. After the third inflation, the angioplasty catheter was withdrawn into the iliac artery, and xylocaine (20 mg) was injected intra-arterially to minimize vascular spasm. Angioplasty was then performed in the contralateral femoral artery. Ten minutes after the last balloon inflation, blood was withdrawn for repeat partial thromboplastin time, xylocaine (20 mg) was administered again, and angiography was repeated.

Rabbits were assigned to four treatment groups: control group (no additional heparin, n=21 arteries), intramural delivery of heparin only (n=20 arteries), subcutaneous heparin only (n=29 arteries), and combined intramural and subcutaneous heparin (n=23 arteries). Arteries that were totally occluded on the initial angiogram were not angioplastied or analyzed. All arteries with successful angioplasty (>10% initial improvement in minimal luminal diameter after angioplasty) were included in the angiographic analysis of restenosis.

Subcutaneous heparin was administered over the entire 28 days (350 units · kg⁻¹ · day⁻¹ injected every 12 hours in a divided dose) beginning within 12 hours of angioplasty.

Intramural delivery of heparin was achieved by use of a 2.5-mm or 2.75-mm porous balloon catheter (USCI Division, C.R. Bard, Inc., Billerica, Mass.) and low (5-atm) inflation pressure. The porous balloon was positioned at the site of previous angioplasty by fluoroscopy and rapidly inflated maintaining 5 atm pressure until a total of 1.5 cm² of heparin (1,500 units) was delivered. The total dose of intramural heparin was limited to 1,500 units because of systemic anticoagulation resulting from intravascular release of heparin at the time of balloon inflation. Thirty-eight arteries with successful balloon angioplasty were treated with the porous balloon catheter. In 25 of 38 arteries (66%), a 2.5-mm balloon was used. The mean heparin infusion time was 39±16 seconds (range, 15–90 seconds, with 23 of 25 arteries [92%] having infusion times between 20 and 65 seconds. With a 2.75-mm balloon, only 6 of 12 arteries (50%) had infusion times of less than 65 seconds, probably representing oversizing of the balloon in this model. After repeat angiography, the right carotid artery was ligated, and the wound was closed. Rabbits were maintained for 28 days after balloon angioplasty on normal rabbit chow, and angiography was repeated before the rabbits were killed.

Detection of Intramural Delivery by Fluoresceinated Heparin

After angiography and bilateral angioplasty in one additional rabbit, fluoresceinated heparin (1.5 cm² [1,000 units/cm² with activity of 170 units/mg], Polysciences, Inc., Warrington, Pa.) was delivered into the angioplastied segment of one femoral artery by a 2.5-mm porous balloon. The contralateral angioplastied femoral artery was not treated with the porous balloon and served as a sham-operated control. Five minutes after repeat angiography, the animal was killed, and both femoral arteries were harvested. The arteries were dissected longitudinally and sectioned transversely in the angioplastied region. Optical images were taken from different planes (z resolution, 0.9 μm) to detect the amount of heparin fluorescence in the plaque, media, and adventitia with a Bio-Rad MRC-600 confocal microscope (Bio-Rad, Cambridge, Mass.). Because the confocal microscope collects signal only from the focal plane (while discarding "blurred" signal), fluorescence in individual layers of the arterial wall can be discriminated.

Killing of Animals and Pressure Perfusion

With the angiographic catheter positioned above the aortoiliac bifurcation, the distal arterial tree was perfused with 10% buffered formaldehyde (100 ml for 15 minutes, 100 mm Hg, 22°C). At the start of perfusion, animals were administered an overdose of sodium pentobarbital. A 4–5-cm segment of each femoral artery was excised, and the proximal and distal ends were marked with silk sutures. The specimens were preserved in 10% formaldehyde for light microscopy.

Quantitative Angiography

All quantitative angiographic measurements were performed by blinded analysis using a computer-assisted system described previously. This blinded analysis was repeated after 3 months. The 35-mm cine frame selected for analysis was mounted in a film holder and projected through a modified Tagarno 35 CX projector (Tagarno A/S, Horsens, Denmark) into a companion videocamera. The video signal was then digitized and stored in a frame grabber board (model DT2861, Data Translation, Inc., Marlboro, Mass.). Analysis was performed using IMAGE-PRO image processing software (Media Cybernetics, Inc., Silver Spring, Md.) in association with an Everest personal computer (model C, System 1700, Fremont, Calif.). Before analysis of the baseline angiogram, the system was calibrated by use of a grid with 1-cm markings placed at the level of the femoral arteries. The minimum luminal diameters (in millimeters) were determined at the sites of focal femoral artery stenosis, of segments proximal and distal to the stenosis, and of the in vivo balloon dimension by placement of a computer-generated line perpendicular to the long axis of the artery. By a prospective definition, the site examined for restenosis was the point of maximal narrowing on the preangioplasty angiogram even if this was not the most severely narrowed site at subsequent time points. Templates were drawn of the preangioplasty angiograms to ensure that the quantitative analyses repeated at 28 days were performed on these same arterial sites. The percent stenosis was defined as the ratio of the minimal luminal diameter to the average of the adjacent segments and was expressed as a decimal fraction. In addition, angiographic measurements were made at 4-mm intervals along the site of balloon angioplasty to...
evaluate the luminal diameter change over the whole angioplastied segment and to mimic the 4-mm sections used for quantitative histopathology. The minimal luminal diameter measured on the 28-day postangioplasty angiogram was compared among the four treatment groups.

Quantitative Histopathology

After the arteries were sectioned at 4-mm intervals, specimens were dehydrated in ethanol and xylene and embedded in paraffin. Sections were stained with hematoxylin and eosin and by the Movat method.22 Histopathological analysis was performed by observers blinded to the treatment. For quantitative histopathological comparisons, the section with the greatest luminal narrowing was identified in each arterial segment. Luminal narrowing was determined by visual estimation (quadrant method)19 and by computerized planimetry. Sections were also assessed with respect to presence of luminal thrombi, extent of plaque tear (up to internal elastic lamina, into media, into adventitia, or complete perforation), mural hemorrhage, severity of medial necrosis (0–3+), extent of inflammatory cell infiltrates (0–3+), and plaque composition. Plaque composition and luminal narrowing were assessed by planimetry using Movat-stained sections from each segment with a CUE-2 image analyzer (Galai Production Ltd., Israel) in association with an Olympus BH-2 microprojection system. After projection of the image, the following areas were traced: external elastic lamina, internal elastic lamina, residual lumen, and plaque components. The areas of plaque occupied by fibrous tissue (cellular and acellular) and foam cells were then computed, as was the percent area that the media occupied of the total area bounded by the external elastic lamina. Luminal narrowing was determined by calculating the difference between the area bounded by the internal elastic lamina and that occupied by plaque (percent cross-sectional area narrowing = 100−[(area of residual lumen/area bounded by internal elastic lamina)×100]).

Statistical Analysis

Data are reported as the number of femoral arteries in each experimental group, and mean±SD values are expressed. Numerical angiographic and histopathological data were analyzed by two-tailed t tests (paired or unpaired, as appropriate). For values that were not normally distributed, a Mann-Whitney U test was used to compare treatment groups. Categorical data were compared by the Fisher exact test. Values of p<0.05 were considered significant.

Results

Partial Thromboplastin Time

All animals received an intra-arterial bolus of heparin immediately before angioplasty. This resulted in prolongation of the partial thromboplastin time from 109±29 seconds at baseline to >200 seconds at the end of the procedure (p<0.0001). The partial thromboplastin time of animals treated chronically with subcutaneous heparin remained prolonged at the midpoint between subcutaneous doses (6 hours after injection) compared with baseline (180±38 seconds, p<0.0001). This confirmed that an anticoagulant dose of heparin was used.

Intramural Delivery of Heparin by Porous Balloon Catheter

To confirm intramural delivery in this atherosclerotic rabbit model, fluoresceinated heparin was delivered by the porous balloon after angioplasty in one animal. By confocal microscopy, the fluorescein was detected to have reached the atherosclerotic plaque and underlying media with localization in the nuclei of the medial and neointimal cells (Figure 2). In the contralateral artery not treated with the porous balloon, minimal background fluorescence was seen.

Angiography

The results of angiography are summarized in Table 1. The individual minimal luminal diameters in the four treatment groups measured before and after balloon angioplasty, after treatment with the porous balloon, and at 28 days after angioplasty are depicted in Figure 3. There was a significant increase in minimal luminal diameter from before to immediately after angioplasty among arteries with successful angioplasty (>10% initial improvement in minimal luminal diameter) (p<0.001) and a significant reduction in minimal luminal diameter at 28 days (p<0.005). The minimal luminal diameter at 28 days after balloon angioplasty was not larger in any treatment group than before angioplasty. The percentage of arteries with successful angioplasty and the ratio of balloon to artery diameter (1.36 to 1) were similar in the four groups (p=NS). Five arteries were occluded at 28 days: two control arteries, two treated with intramural heparin only, and one treated with intramural and subcutaneous heparin. The minimal luminal diameter in occluded arteries was defined to be zero. No arteries occluded acutely after treatment with the porous balloon, no perforations were apparent angiographically, and porous balloon size (2.5 versus 2.75 mm) did not affect angiographic (or pathological) outcome. The two blinded readings of the postangioplasty and 28-day luminal diameters in this study correlated with values of r=0.94 and r=0.84, respectively.

Analysis of Angiographic Restenosis

Comparisons of angiograms among the four treatment groups showed similar minimal luminal diameters before as well as immediately after balloon angioplasty (Table 1). The minimal luminal diameters measured immediately before and immediately after intramural delivery of heparin by porous balloon were also similar (1.59±0.29 versus 1.65±0.32 mm, p=NS) (Table 1). Angiographic restenosis, defined as the change in minimal luminal diameter (in millimeters) from immediately after to 28 days after angioplasty, was reduced by 45% in arteries treated with subcutaneous heparin compared with control arteries. The reduction in minimal luminal diameter during the 28 days after angioplasty was 0.32±0.18 mm in the subcutaneous heparin only group compared with 0.58±0.34 mm in control arteries (p<0.01) (Figure 4). The second blinded reading gave similar results (reduction in minimal luminal diameter of those receiving subcutaneous heparin, 0.23±0.33 mm versus control arteries, 0.68±0.47 mm,
FIGURE 2. Confocal scanned fluorescence micrograph of an atherosclerotic femoral artery treated with balloon angioplasty followed by intramural delivery of fluoresceinated heparin. Note the intense fluorescence in the plaque (P) and media (M). L, lumen.

*p<0.01). The increase in percent stenosis (Figure 5) during the 28 days after angioplasty was likewise less in arteries treated with subcutaneous heparin compared with controls (0.16±0.08 versus 0.31±0.18, *p<0.01). Figure 4 illustrates that only 1 of 15 arteries (7%) in the subcutaneous-only group restenosed by more than 0.5 mm compared with 11 of 18 (61%) in control arteries (*p=0.001). This reduction in angiographic restenosis remained significant when the two total occlusions (at 28 days) in the control group were excluded from analysis. In an expanded analysis that included all patent arteries after angioplasty, angiographic restenosis (defined as the reduction in luminal diameter) was significantly less in the subcutaneous heparin-only group compared with control arteries (-0.24±0.24 mm [n=26] versus -0.57±0.39 mm [n=21], *p<0.001).

TABLE 1. Angiographic Minimal Luminal Diameter and Percent Stenosis in Arteries With Successful Angioplasty

<table>
<thead>
<tr>
<th></th>
<th>Control (n=18)</th>
<th>Intramural only (n=20)</th>
<th>SC only (n=15)</th>
<th>SC and intramural heparin (n=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal luminal diameter (mm)</td>
<td>1.12±0.26</td>
<td>1.22±0.25</td>
<td>1.22±0.24</td>
<td>1.14±0.18</td>
<td>NS</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.57±0.28</td>
<td>1.66±0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Postangioplasty</td>
<td></td>
<td></td>
<td>1.56±0.22</td>
<td>1.54±0.24</td>
<td></td>
</tr>
<tr>
<td>Post–porous balloon</td>
<td></td>
<td></td>
<td>1.75±0.39</td>
<td>1.56±0.22</td>
<td>NS</td>
</tr>
<tr>
<td>28 Days</td>
<td>0.99±0.45</td>
<td>1.05±0.48</td>
<td>1.24±0.24*</td>
<td>1.12±0.48</td>
<td>*0.05 vs. control</td>
</tr>
<tr>
<td>Δ Post to 28 days</td>
<td>-0.58±0.34</td>
<td>-0.61±0.54</td>
<td>-0.32±0.18*</td>
<td>-0.43±0.41</td>
<td>*&lt;0.01 vs. control</td>
</tr>
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</table>

Percent stenosis

<table>
<thead>
<tr>
<th></th>
<th>Control (n=18)</th>
<th>Intramural only (n=20)</th>
<th>SC only (n=15)</th>
<th>SC and intramural heparin (n=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preangioplasty</td>
<td>0.41±0.11</td>
<td>0.34±0.14</td>
<td>0.39±0.07</td>
<td>0.42±0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Postangioplasty</td>
<td>0.16±0.15</td>
<td>0.09±0.25</td>
<td>0.21±0.11</td>
<td>0.21±0.16</td>
<td>NS</td>
</tr>
<tr>
<td>28 Days</td>
<td>0.47±0.23</td>
<td>0.41±0.30</td>
<td>0.37±0.15</td>
<td>0.44±0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Δ Post to 28 days</td>
<td>0.31±0.18</td>
<td>0.33±0.29</td>
<td>0.16±0.08*</td>
<td>0.23±0.26</td>
<td>*&lt;0.01 vs. control</td>
</tr>
</tbody>
</table>

SC, subcutaneous heparin. Values are expressed as mean±SD in the four treatment groups. n, Numbers of arteries in each group. Luminal diameters and change in luminal diameter (Δ) are expressed in millimeters. Percent stenosis is expressed as a decimal. Values are given before angioplasty (pre), after angioplasty (post), after porous balloon (post–porous balloon), and 28 days after angioplasty (28 days).
ysis of all patent arteries after angioplasty confirmed less angiographic restenosis (as measured by the change in percent stenosis) in the subcutaneous heparin-only group compared with control arteries (0.12±0.11 [n=26] versus 0.30±0.21 [n=21], p<0.001).

Intramural heparin therapy did not reduce angiographic restenosis (Table 1, Figures 4, 5). Arteries in the intramural-only group narrowed by 0.61±0.54 mm compared with 0.58±0.34 mm in control arteries (p=NS). The change in percent stenosis from immediately after angioplasty to 28 days after angioplasty was also similar in the two groups (0.33±0.29 versus 0.31±0.18, p=NS).

Intramural combined with subcutaneous heparin therapy showed a trend toward decreasing angiographic restenosis. Arteries in this group narrowed by 0.43±0.41 mm compared with 0.58±0.34 mm in control arteries (p=0.22). The change in percent stenosis from immediately after angioplasty to 28 days after was also somewhat less in this group (0.23±0.26 versus 0.31±0.18, p=0.26).

Angiographic Analysis at 4-mm Intervals

Angiograms were also analyzed by a method that mimics as closely as possible the 4-mm sections used for quantitative histopathology. In this analysis, the arterial segment dilated was identified on the 28-day angiogram, the luminal diameter was measured at 4-mm intervals, and the smallest diameter was identified. Comparison of the narrowest diameter between the four treatment groups showed no statistically significant differences (0.92±0.45, 0.97±0.38, 1.13±0.22, and 1.02±0.48 mm, respectively, Figure 6). There was a trend to less arterial narrowing in the group receiving chronic subcutaneous heparin, but this was not significant (1.13±0.22 versus 0.92±0.45, p=0.10). This analysis is concordant with the quantitative histopathology.

Histopathology

Morphometric analysis. Quantitative histopathological analysis using computerized planimetry of arteries 28 days after angioplasty showed similar degrees of
luminal cross-sectional area narrowing by atherosclerotic plaque among the four treatment groups (control arteries, 47±26%; intramural only, 55±19%; subcutaneous only, 45±18%; subcutaneous and intramural, 42±23%; p=NS) (Table 2). The cross-sectional area narrowing by plaque for individual arteries is summarized in Figure 7. The quadrant method of analysis similarly showed no significant differences in luminal narrowing by plaque among groups. The absolute lumen areas, plaque areas, and plaque/media ratios were not significantly different among the four treatment groups (Table 2).

**Vascular injury.** There were no significant differences in the overall frequency or severity of plaque tears

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**TABLE 2. Morphometric and Histopathological Findings 28 Days After Angioplasty**

<table>
<thead>
<tr>
<th>Morphometric</th>
<th>Control (n=21)</th>
<th>Intramural only (n=18)</th>
<th>SC only (n=29)</th>
<th>SC and intramural (n=23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of cross-sectional area narrowed by plaque</td>
<td>47±26</td>
<td>55±19</td>
<td>45±18</td>
<td>42±23</td>
<td>NS</td>
</tr>
<tr>
<td>Lumen area×10³ μm²</td>
<td>877±479</td>
<td>837±819</td>
<td>922±443</td>
<td>947±459</td>
<td>NS</td>
</tr>
<tr>
<td>Plaque area×10³ μm²</td>
<td>794±484</td>
<td>1,171±1,111</td>
<td>789±477</td>
<td>702±491</td>
<td>NS</td>
</tr>
<tr>
<td>Plaque/media ratio</td>
<td>1.77±1.26</td>
<td>2.84±3.47</td>
<td>2.34±2.38</td>
<td>1.63±1.21</td>
<td>NS</td>
</tr>
<tr>
<td>Plaque components*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam cells, %</td>
<td>10±22</td>
<td>11±20</td>
<td>2±4*</td>
<td>4±10</td>
<td>*0.04 SC vs. control</td>
</tr>
<tr>
<td>Fibrous tissue, %</td>
<td>90±22</td>
<td>89±20</td>
<td>98±4*</td>
<td>96±10</td>
<td>*0.04 SC vs. control</td>
</tr>
</tbody>
</table>

**Histological**

| Luminal thrombi, no. | 1 | 0 | 0 | 0 | ... |
| Plaque tears, no. (%) | | | | | |
| Up to IEL | 1 (5) | 0 | 5 (17) | 0 | NS |
| Into media | 10 (48) | 11 (61) | 17 (59) | 17 (74) | NS |
| Into adventitia | 6 (29) | 6 (33) | 6 (21) | 4 (17) | NS |
| Perforation | 1 (5) | 1 (6) | 0 | 1 (4) | NS |
| Total | 18 (86) | 17 (94) | 27 (93) | 23 (100) | NS |
| Mural hemorrhage, no. (%) | 13 (62) | 8 (44) | 18 (62) | 14 (61) | NS |
| Severe medial necrosis, no. (%) having 2+ or 3+/3+ | 12 (57) | 13 (72) | 20 (69) | 12 (52) | NS |
| Extensive inflammatory cell infiltrates, no. (%) having 2+ or 3+/3+ | 10 (48) | 10 (56) | 13 (45) | 16 (70) | NS |

SC, subcutaneous; n, no. of arteries; IEL, internal elastic lamina. Values are expressed as mean±SD.
*Each number represents the mean percent±SD of plaque area occupied by the components listed.
between the treatment groups and no differences in frequency of mural hemorrhage, severe medial necrosis, or extensive inflammatory cell infiltration (Table 2, Figure 8). Mural perforation occurred in one artery of the control group, in one treated with intramural heparin only, in one with subcutaneous and intramural heparin, and in none receiving subcutaneous heparin only. Treatment with the porous balloon was not associated with increased vascular damage. Luminal thrombus was present at the site of angioplasty at 28 days in only one control artery but in none of the other arteries.

**Plaque composition.** By planimetric analysis of Movat-stained sections of femoral arteries at sites of balloon angioplasty, the plaques consisted primarily of cellular and acellular fibrous tissue (94±15%), with a much smaller mean percent of plaque area occupied by foam cells (6±15%) (Table 2, Figure 8). Comparisons between the four treatment groups showed a significantly lower percentage of foam cells in arteries treated with subcutaneous heparin. Arteries treated with chronic subcutaneous heparin had a lower percentage of foam cells (2±4%) than either controls (10±22%, p<0.05) or arteries treated with intramural delivery of heparin only (11±20%, p<0.05) but had a similar percentage to those treated with intramural and subcutaneous heparin (4±10%, p=0.35). Calcific deposits were absent.

**Discussion**

We conclude that although heparin can be effectively delivered into an atherosclerotic plaque, this "site-specific" therapy does not reduce restenosis in our model. Chronic administration of subcutaneous heparin for 28 days after angioplasty resulted in modest reduction in angiographic restenosis, but this treatment did not improve luminal diameter compared with preangioplasty, and quantitative histopathology showed no reduction in luminal cross-sectional area narrowing by plaque or in plaque area. Because previous investigators had shown more marked reductions in plaque area with heparin treatment in other models, our data emphasize that heparin's effect on restenosis may depend on the model studied, the presence or absence of preexisting atherosclerotic plaque, and on the extent of injury.

Although previous studies have suggested that "site-specific" therapy with heparin might reduce smooth muscle cell migration and proliferation after vascular injury, in our study quantitative angiography showed no reduction in restenosis after direct intramural delivery of heparin via a porous balloon catheter. Quantitative histopathological analysis by computerized planimetry also showed no effect on luminal cross-sectional area narrowing by plaque at 28 days. There are several potential explanations for the lack of benefit of intramural heparin administration in this model compared with previous reports. Adventitial heparin delivery (anticoagulant or nonanticoagulant) via surgically placed heparin-impregnated polymers has been shown to reduce intimal hyperplasia after balloon injury of normal rat carotid artery. It is known, however, that the arterial response to injury of an abnormal artery (second injury) can be significantly different, with thrombosis playing a more important role.23 Additionally, atherosclerotic models may more closely reproduce the varied cell types present in human atherosclerotic plaques at the time of balloon angioplasty. We investigated the use of a clinically relevant method of delivering heparin into the arterial wall by use of a catheter-based system. It is possible, however, that the 5-atm porous balloon inflation used for the local administration of heparin resulted in additional arterial injury not apparent by angiography or light microscopy. We cannot exclude that increased arterial injury resulting from this system masked any relative benefit resulting from the local administration of heparin. The present studies using fluoresceinated heparin confirmed previous reports15,16 that the porous balloon can reliably deliver heparin into the arterial wall. Although the final concentration of heparin in the wall was not directly measured in this chronic animal model, the maximal dose of heparin was used that did not result in excessive systemic anticoagulation. It is of interest that fluoresceinated heparin was seen throughout the atherosclerotic plaque and underlying media with evidence of localization of heparin to the nuclei of medial and neointimal cells (Figure 2). There is uncertainty regarding the permeability of normal smooth muscle cells to heparin.20 In this study, light microscopy at 28 days suggested extensive medial necrosis associated with balloon angioplasty in all treatment groups (Table 2). It is possible that penetration of fluoresceinated heparin into smooth muscle cells resulted from cellular damage after balloon angioplasty. The localization of heparin to the cellular nucleus is thought to occur by interaction with a chromatin-associated inner histone.24

We hypothesized that heparin, possibly because of its antithrombin, antithrombotic, and antiproliferative effects, would reduce restenosis after balloon angioplasty. Thrombin activity is known to be elevated for up to 10 days after vascular injury.25 Recently, the synthesis of thrombin receptors by endothelial and smooth muscle cells has been reported.26 Because thrombin is known to inhibit endothelial growth, to stimulate smooth muscle cell proliferation,27 and to promote monocyte adhesion to the vascular surface,28 heparin could potentially limit restenosis via its antithrombin III–mediated inhibition.
**FIGURE 8.** Movat-stained histological sections of femoral arteries of rabbits killed 28 days after balloon angioplasty. Panel A, control artery (no further treatment after angioplasty); panels B and C, intramural delivery of heparin by porous balloon catheter immediately after angioplasty. Disruption of the internal elastic lamina (IEL) is seen in panels A and B with its edges rolled back over itself as is typically seen after balloon angioplasty. In panel A, mural hemorrhage (H) can be seen extending from the intimal plaque, through the defect in the internal elastic lamina, and into the outer media. Panel C, total occlusion of the lumen by plaque after intramural delivery of heparin. The plaques consisted primarily of fibrous tissue (FT) (cellular and acellular) and foam cells (FC). By planimetry, no significant differences were found in mean luminal cross-sectional area narrowing by plaque between the two groups. L, lumen. Original magnification: Panel A, ×90; panel B, ×150; panel C, ×50.
of thrombin. In our study, quantitative computer-assisted angiography did show less restenosis in arteries receiving chronic subcutaneous heparin compared with controls. However, none of the groups had significant improvement in luminal diameters at 28 days compared with preangioplasty. Restenosis in humans is best defined as the change in angiographic minimal luminal diameter from immediately after balloon angioplasty to time of follow-up. Therefore, we used this prospective definition as the primary angiographic endpoint in this and previous studies. To account for apparent differences in the angiographic and histological results in this study, we mimicked histological sectioning by analyzing each 28-day angiogram at 4-mm intervals along the arterial segment undergoing balloon angioplasty. With this technique, no significant differences were seen among the four treatment groups. Consistent with this finding, morphometric analysis of histological sections taken at 4-mm intervals showed similar percent luminal cross-sectional narrowing by atherosclerotic plaque among the four treatment groups when examined at the single 28-day time point. Furthermore, no significant differences were seen in plaque or media area or in the plaque-to-media ratio. As was previously seen when the more potent thrombin inhibitor recombinant desu-phathoruridin was used, the foam cell content of plaque was reduced by chronic subcutaneous heparin. However, the inhibition of plaque growth with recombinant desu-phathoruridin documented histopathologically was not seen with heparin at the dose used, possibly because heparin is a less potent antithrombin agent.

Limitations of the Study

Optimal heparin dosing to be used in balloon angioplasty in humans or animals is unknown. Because an intravenous heparin bolus was necessary to avoid acute catheter-related thrombosis, we cannot exclude the possibility that the heparin bolus used in controls had some effect on late restenosis. Although the subcutaneous dose used here resulted in “therapeutic anticoagulation” (prolongation of the activated partial thromboplastin time to >1.5 times control between doses), it is known that even very high doses of heparin do not completely inhibit the mural thrombosis associated with deep arterial injury. We did not specifically measure the heparin levels achieved locally in the atherosclerotic plaque after chronic subcutaneous administration of heparin, but instead decided to investigate the use of clinically applicable doses of subcutaneous heparin. Dosing of intramural heparin by use of the porous balloon was limited by the systemic anticoagulation resulting from release of heparin intra-arterially during low-pressure balloon inflation. We decided that arteries treated with the porous balloon would receive one additional (low-pressure) balloon inflation (compared with controls), because this would be a clinically relevant method. No additional arterial injury was observed angiographically or histologically by this technique. Previous investigators have documented that heparin can be reliably delivered into both normal and atherosclerotic arterial wall by the porous balloon. Our single experiment using fluoresceinated heparin was done to document heparin delivery after balloon angioplasty in our atherosclerotic model.

No animal model of balloon angioplasty exactly reproduces the experience in humans. Human restenosis trials, however, are enormously expensive and fail to answer many mechanistic questions regarding vascular injury and healing. Animal models allow for the systematic comparison of angiographic and histological consequences of balloon injury. Because injury of normal arteries is known to be significantly less thrombogenic than “second injury” of atherosclerotic arteries, the model studied in this investigation may be especially well suited to the investigation of antithrombotic therapies. The atherosclerotic lesions produced in this model were similar in morphological characteristics to those seen in human coronary arteries consisting primarily of fibrous tissue with few foam cells before angioplasty. Nevertheless, this study suggests that therapies found effective against intimal hyperplasia in one model (normal arteries) may be less effective in models with preexisting atherosclerotic plaque or in humans with coronary artery disease.

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