Effectiveness of Recombinant Desulphatohirudin in Reducing Restenosis After Balloon Angioplasty of Atherosclerotic Femoral Arteries in Rabbits

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**Background.** The effectiveness of balloon angioplasty is limited by a restenosis rate of approximately 30%. Recombinant desulphatohirudin (r-hirudin [CGP 39393]) has been found to be highly effective in preventing acute platelet-rich thrombosis after deep arterial injury as compared with heparin.

**Methods and Results.** This study evaluated the effect of intravenous r-hirudin, a selective inhibitor of thrombin, on restenosis after balloon angioplasty in 29 rabbits. Focal femoral atherosclerosis was induced by air desiccation endothelial injury followed by a 2% cholesterol diet for 1 month. At angioplasty (2.5-mm balloon with three 60-second, 10-atm inflations 60 seconds apart), the rabbits received heparin (150 units/kg bolus, n=16) or r-hirudin (1 mg/kg bolus followed by infusions of 1 mg/kg for the first hour and 0.5 mg/kg for the second hour, n=13). Angiograms performed before and after angioplasty and before death were analyzed quantitatively by a blinded observer. Rabbits were killed 2 hours (n=14) or 28 days (n=15) after angioplasty. Femoral arteries were fixed in situ by perfusion of 10% formaldehyde at 100 mm Hg. The mean luminal diameter of the arteries with successful angioplasty (≥20% increase in luminal diameter) in rabbits treated with heparin (n=8 arteries) increased from 1.18±0.29 mm before angioplasty to 1.86±0.24 mm immediately after angioplasty (p<0.001) and decreased to 0.94±0.69 mm (p=0.0004) at 28 days after angioplasty. In rabbits treated with r-hirudin (n=11 arteries), the mean luminal diameter increased from 1.14±0.17 mm before angioplasty to 1.68±0.20 mm immediately after angioplasty (p<0.001) and decreased to 1.37±0.47 mm (p=0.01) at 28 days after angioplasty. The mean reduction in luminal diameter by angiography was less in the r-hirudin–treated group than in the heparin-treated group (0.30±0.33 versus 0.92±0.61 mm, p=0.01). Blinded planimetric analysis of stained histological sections of the femoral arteries also showed less cross-sectional area narrowing by plaque in rabbits treated with r-hirudin compared with those treated with heparin (22±16% versus 48±29%, p=0.01). Both groups had similar numbers of arteries with histological evidence of balloon-induced plaque tear (12 of 13 versus 13 of 15).

**Conclusions.** Rabbits receiving r-hirudin at the time of experimental balloon angioplasty had significantly less restenosis by angiography and by quantitative histopathology than rabbits receiving heparin. (*Circulation* 1991;84:232–243)

Despite the widespread use of balloon angioplasty as an alternative to surgical revascularization in patients with significant coronary artery disease, its effectiveness remains limited by an approximately 30% restenosis rate within 6 months after angioplasty.1–6 The cause of the restenosis remains undefined. It is thought to result from a complex interaction of biological processes includ-

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ing platelet deposition and thrombus formation, release of vasoactive and mitogenic factors, and migration and proliferation of smooth muscle cells in the intima of the dilated arterial segment.7-9

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Although platelet factors10,11 promote growth after mechanical disruption of an artery, the important role of thrombin in acute platelet-rich thrombosis12,13 and vascular healing14 has recently been highlighted. Thrombin is generated in large amounts at the time of arterial injury and is amplified by the prothrombinase complex.15 It has been shown to be a potent stimulus for platelet activation16-18 and also has mitogenic potential.19-27 Recombinant hirudin (r-hirudin), a specific and potent inhibitor of thrombin,28-30 is highly effective in preventing acute platelet-rich thrombosis after deep arterial injury as compared with heparin.12,13 We hypothesized that r-hirudin treatment, because of its potent antithrombin properties, would significantly reduce restenosis after experimental balloon angioplasty compared with bolus heparin treatment.

Methods

The study design is summarized in Figure 1. Briefly, femoral artery atherosclerosis was induced in 29 rabbits. The rabbits were randomly assigned to bolus heparin or r-hirudin therapy. Balloon angioplasty was performed, and the rabbits were killed at either 2 hours or 28 days after angioplasty. Angiographic, histological, and morphometric analyses were then performed. The model used was first described by LeVeen et al,31 and the methodology used in this paper has been previously described in detail.32

Induction of Focal Atherosclerosis

Twenty-nine male New Zealand White rabbits weighing 4.1±0.4 kg (range, 3.2-4.6 kg) were anesthetized with 50 mg/kg ketamine and 5 mg/kg xylazine by intramuscular injection. Atherosclerosis was induced in femoral artery segments (1-2 cm in length) by air desiccation endothelial injury; rabbits were then fed a 2% cholesterol, 6% peanut oil diet for 1 month.32-34 We have previously shown32 that cholesterol levels increased approximately 20-25-fold after 1 month on this diet. After angioplasty, the rabbits received standard rabbit chow for 28 days before death. The rabbits were housed according to Animal Welfare Act specifications, and all surgical procedures were performed using a sterile technique and general anesthesia.

Angioplasty

Balloon angioplasty was performed 37±6 days (range, 28-56 days) after endothelial injury. Via a right common carotid arteriotomy, a 5F Berman angiographic balloon catheter (Arrow International, Inc., Reading, Pa.) was inserted and advanced into the descending aorta. A 5F vascular sheath was advanced over the catheter. The angiographic bal-

STUDY DESIGN

FIGURE 1. Flow diagram of study protocol. Twenty-nine New Zealand White rabbits had femoral artery atherosclerosis induced by air desiccation endothelial injury followed by a high cholesterol diet. At time of balloon angioplasty, rabbits were randomly assigned to heparin or recombinant desulphated hirudin therapy. Balloon angioplasty was performed using a 2.5-mm balloon and three 60-second, 10-atm inflations. Rabbits were killed at 2 hours and 28 days after angioplasty for angiographic, histological, and morphometric analysis of angioplastied femoral arteries.

loon catheter was then positioned two vertebral spaces above the aortic bifurcation. Blood was drawn for baseline activated partial thromboplastin time, and 16 rabbits were randomly assigned to receive an intra-arterial bolus of 150 units/kg heparin (heparin sodium injection, 1,000 USP units/ml, porcine intestinal mucosa, Solopak Laboratories, Franklin Park, Ill.). Thirteen other rabbits received r-hirudin (CGP 39393, Lot rHH5-7018/No. A16, CIBA-GEIGY Ltd., Basel, Switzerland) as a 1 mg/kg i.v. bolus, followed by a constant infusion of 1 mg/kg i.v. for the first hour and 0.5 mg/kg i.v. for the second hour after balloon angioplasty. After heparin or r-hirudin administration, a baseline angiogram of the iliac and femoral arteries was obtained using a Siemens Optilux Angiographic System (model 1179878VS048, Siemens Aktiengesellschaft, Munich), and images were recorded on 35-mm cineradiographic film using 5 ml Hypaque 76 (diatrizoate meglumine and diatrizoate sodium injection USP, Winthrop-Breon Laboratories, New York) diluted with 5 ml sterile saline. The angiographic catheter was replaced by a 0.014-in. guide
wire (Advanced Cardiovascular Systems [ACS], Inc., Temecula, Calif.) and an ACS balloon dilatation catheter. The angioplasty catheter was advanced across the femoral stenosis, and the correct balloon placement was verified using fluoroscopy and the previously placed metal clips. Using a 2.5-mm balloon catheter, three 60-second, 10-atm balloon inflations 1 minute apart were performed under fluoroscopic vision using a hand inflator (ACS Indeﬂator, Advanced Cardiovascular Systems, Inc., Mountain View, Calif.). The in vivo balloon dimension was recorded using cineangiography. The contralateral femoral stenosis was dilated in the same manner. The angioplasty catheter was then replaced by the angio-
graphic catheter positioned above the aortic bifurcation. Blood was then drawn for repeat partial thromboplastin time. A postangioplasty angiogram was performed 10 minutes after the last balloon inﬂation. A grid with 1-cm markings was used as an internal calibration standard. After removal of the angio-
plasty catheter and vascular sheath, the right carotid artery was ligated, and the wound was closed.

Rabbits killed 2 hours after angioplasty (n = 14) had a follow-up angiogram immediately before death. Rabbits maintained for 28 days after balloon angioplasty (n = 15) had repeat angiography before death via the left carotid artery using the above technique.

**Pressure Perfusion and Specimen Preparation**

Fourteen (eight heparin-treated and six r-hirudin-
treated) rabbits were killed 2 hours after balloon angioplasty, and 15 (eight heparin-treated and seven r-hirudin-treated) rabbits were maintained for 28 days before death. The distal arterial tree was perfused with 10% buffered formaldehyde solution (100 ml for 15 minutes at 100 mm Hg35 and 22°C, Lyne Laboratories, Inc., Stoughton, Mass.). At the start of perfusion, the rabbits were administered an overdose of Nembutal. A 4–5-cm segment of femoral artery with a surrounding cuff of muscle was excised bilat-
erally with the proximal and distal ends marked with silk sutures. The specimens were preserved in 10% formaldehyde for light microscopy.

**Data Analysis**

**Quantitative angiography.** All quantitative angiographic measurements were made using a computer-
assisted system described previously.36 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 the companion videocamera (Tagarno A/S). The video signal produced by the camera was then digi-
tized and stored in a frame-grabber board (model DT2861, Data Translation, Inc., Marlboro, Mass.). Analysis was performed using image-processing soft-
ware (IMAGE-PRO, Media Cybernetics, Inc., Silver Spring, Md.) in association with a personal computer (System 1700, model C, Everex, Fremont, Calif.). Before analysis of the baseline angiogram, the system was calibrated using a grid with 1-cm markings placed at the level of the femoral arteries. Calibration of the center 1-cm square to screen units was then per-
formed. The minimum luminal diameters (mm) of the focal femoral artery stenoses, of the segment proximal to and distal to the stenoses, and of the in vivo balloon dimension were determined by placing a computer-
generated line perpendicular to the long axis of the vessel. The percent stenosis was deﬁned as the ratio of the minimal luminal diameter to the average of the adjacent segments. Templates were drawn of the preangioplasty and postangioplasty angiograms of the chronic rabbits to ensure that quantitative analysis was performed on the same vessel sites after 28 days. The intraobserver variability of this system was r = 0.93 using 40 measured femoral artery segments.

**Quantitative histopathology.** The arterial segments were sectioned transversely at 5-mm intervals, dehy-
drated in ethanol and xylene, and embedded in parafﬁn. Sections (5 μm thick) were stained by the Movat method.37 Histopathologic analysis was performed by observers blinded to treatment and time of death.

For quantitative histopathologic comparisons, the section with the greatest luminal narrowing was iden-
tiﬁed in each arterial segment. Luminal narrowing was determined by visual estimation and by planimetry. The degree of luminal narrowing by visual analysis was assessed by subdividing the circle formed by the internal elastic lamina into four equal quadrants.38 These quadrants corresponded to the four degrees of reduction of luminal cross-sectional area: 0–25%, 26–50%, 51–75%, and 76–100%, with the latter being subdivided further (76–95% and 96–100%). Sections were also categorized with re-
spect to the presence of luminal thrombi, eccentricity of the lumen, extent of plaque tear (to internal elastic lamina, into the media, into the adventitia, or complete perforation), hemorrhage into the plaque, ex-
tent of medial necrosis and inﬂammatory cell inﬁl-
trates, and plaque composition.

Plaque composition and luminal narrowing were assessed quantitatively by planimetry as follows: Movat-stained sections37 from each segment were projected onto white paper using a Tri-simpex micro-
projector (Bausch and Lomb, Rochester, N.Y.). The image was magniﬁed at ×40, and the following areas were traced: external elastic lamina (corresponding to the outer border of the media), internal elastic lamina (potential lumen minus total area of athero-
sclerotic plaque), residual lumen, ﬁbrous tissue (cel-

eral and acellular—primarily collagenous with ﬁbro-
blasts, some elastic ﬁbers and smooth muscle cells, and rich in mucopolysaccharides), calciﬁc deposits (granular, brown-stained areas in Movat-stained sec-

tions), and foam cells. After tracing these compo-

ents, the relative area occupied by each was deter-
mined by reoutlining these areas using a Micro Digi-pad (GTCO Corp., Columbia, Ind.) and stylus (model 1212, GTCO) in association with the Mac-

Morphometric software package adapted to a Macintosh SE computer.39
TABLE 1. Luminal Diameters of Rabbits Before, Immediately After, and 2 Hours After Angioplasty

<table>
<thead>
<tr>
<th>Arteries patent immediately after angioplasty</th>
<th>Arteries with initial success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin (n=12)</td>
<td>Heparin (n=11)</td>
</tr>
<tr>
<td>(n=9)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>Luminal diameters (mm)</td>
<td></td>
</tr>
<tr>
<td>Before angioplasty</td>
<td></td>
</tr>
<tr>
<td>1.21±0.27</td>
<td>1.13±0.23</td>
</tr>
<tr>
<td>Immediately after angioplasty</td>
<td></td>
</tr>
<tr>
<td>1.60±0.30</td>
<td>0.96±0.23</td>
</tr>
<tr>
<td>1.61±0.31</td>
<td></td>
</tr>
<tr>
<td>2 hours after angioplasty</td>
<td></td>
</tr>
<tr>
<td>1.68±0.32</td>
<td></td>
</tr>
<tr>
<td>CSA (%)</td>
<td></td>
</tr>
<tr>
<td>25±20</td>
<td></td>
</tr>
<tr>
<td>(n=11)</td>
<td>(n=11)</td>
</tr>
</tbody>
</table>
| Values are mean±1 SD. Heparin, rabbits treated with heparin at time of angioplasty; Hirudin, rabbits treated with recombinant desulphatohirudin at time of angioplasty; NS, not significant; CSA, cross-sectional area narrowed by plaque 2 hours after angioplasty. **p<0.001 and *p<0.01 vs. within-group value before angioplasty.**

Statistical Analysis

Data are reported as the number of femoral arteries in each experimental group and expressed as mean±1 SD. The angiographic and histopathologic data were analyzed using Student's t test to evaluate two-tailed levels of significance. Paired and unpaired tests were used as appropriate. For values that were not normally distributed, a Mann-Whitney U test was used to compare treatment groups. Comparison of categorical data was made using the two-tailed Fisher's exact test. A value of p≤0.05 was considered significant.

Results

Partial Thromboplastin Times

Both treatment regimens resulted in therapeutic prolongation of the partial thromboplastin time to greater than 1.5–2.0 times control. In rabbits treated with heparin, the partial thromboplastin time was 87±25 seconds at baseline, increased to 189±31 seconds immediately after angioplasty, and was 152±4 seconds 2 hours after angioplasty. In the r-hirudin–treated group, the partial thromboplastin time was 86±24 seconds at baseline, increased to greater than 200 seconds immediately after angioplasty, and was greater than 200 seconds after 2 hours.

Luminal Diameters Before, Immediately After, and 2 Hours After Angioplasty

The angiographic data of rabbits killed 28 days after angioplasty are summarized in Table 2. The individual angiographic luminal diameters of the angioplasty site before, after, and 28 days after angioplasty for the heparin-treated and r-hirudin–treated groups are illustrated in Figure 2.

In the heparin-treated group, 11 of 13 arteries were patent immediately after angioplasty, and eight of 11 (73%) had initial angiographic success. Figure 3 illustrates a series of angiograms from a heparin-treated rabbit before, after, and 28 days after angioplasty, demonstrating initial success with subsequent restenosis.

In the r-hirudin–treated group, all 13 arteries were patent immediately after angioplasty. Eleven of 13 r-hirudin–treated arteries (85%) had initial angiographic success.

Evaluation of restenosis (Table 2). At baseline, there was no difference in the mean luminal diameters between the two treatment groups. Likewise, the mean luminal diameters were similar between the heparin-treated and r-hirudin–treated groups immediately after angioplasty. Restenosis, defined as the millimeter change in luminal diameter from immediately after angioplasty to 28 days after angioplasty, was significantly less in the r-hirudin–treated group than in the heparin-treated group. Among all arteries that were patent after angioplasty, the mean luminal diameter of those treated with heparin decreased by 0.77±0.61 mm, whereas those treated with r-hirudin decreased by only 0.25±0.40 mm (p=0.05). Figure 4 illustrates the subsets with initial angiographic success. In these subsets, the degree of restenosis is significantly less in the r-hirudin–treated group (0.30±0.33 mm) compared with the heparin-treated group (0.92±0.61 mm, p=0.01). Four of eight (50%) arteries in the heparin-treated group had a greater than 1.0 mm decrease in luminal diameter compared with none of the 11 in the r-hirudin–treated group (p=0.02). The change in percent stenosis is expressed as a decimal fraction (for example, a reduction in percent stenosis from 35% to 10% is ex-
pressed as a 0.25 change). Analysis of the change in percent stenosis from immediately after angioplasty to 28 days after angioplasty for all arteries patent after angioplasty likewise demonstrated less restenosis in the r-hirudin–treated group. The change in percent stenosis was 0.46±0.38 in the heparin-treated group versus 0.14±0.23 in the r-hirudin–treated group (p=0.04). In the subsets with initial angiographic success, the change in percent stenosis was 0.53±0.38 in the heparin-treated subset versus 0.18±0.18 in the r-hirudin–treated subset (p=0.02).

### Histopathology

**Luminal narrowing.** Examination of the arterial segments (n=50) after angioplasty showed that 24 (48%) were narrowed 0–25% in luminal cross-sectional area by atherosclerotic plaque; 18 (36%) were narrowed 26–50%; five (10%) were narrowed 51–75%; and 3 (6%) were narrowed more than 75%. By blinded planimetric analysis, the mean percent luminal cross-sectional area narrowing by atherosclerotic plaque of all the arteries was 31±25%.

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**TABLE 2. Luminal Diameters of Rabbits Before, Immediately After, and 28 Days After Angioplasty**

<table>
<thead>
<tr>
<th>Arteries patent immediately after angioplasty</th>
<th>Arteries with initial success</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal diameter (mm)</td>
<td></td>
</tr>
<tr>
<td>Heparin (n=11)</td>
<td>Heparin (n=8)</td>
</tr>
<tr>
<td>Heparin (n=13)</td>
<td>Hirudin (n=11)</td>
</tr>
<tr>
<td>Before angioplasty</td>
<td></td>
</tr>
<tr>
<td>1.24±0.36</td>
<td>1.18±0.29</td>
</tr>
<tr>
<td>1.61±0.2</td>
<td>1.14±0.17</td>
</tr>
<tr>
<td>Immediately after angioplasty</td>
<td></td>
</tr>
<tr>
<td>1.77±0.36*</td>
<td>1.86±0.24*</td>
</tr>
<tr>
<td>1.61±0.31*</td>
<td>1.68±0.20*</td>
</tr>
<tr>
<td>28 days after angioplasty</td>
<td></td>
</tr>
<tr>
<td>1.00±0.75†</td>
<td>0.94±0.69†</td>
</tr>
<tr>
<td>1.36±0.43†</td>
<td>1.37±0.47†</td>
</tr>
<tr>
<td>ΔDiameter</td>
<td></td>
</tr>
<tr>
<td>0.77±0.61</td>
<td>0.92±0.61</td>
</tr>
<tr>
<td>0.25±0.40</td>
<td>0.30±0.33</td>
</tr>
<tr>
<td>Stenosis (%)</td>
<td></td>
</tr>
<tr>
<td>Before angioplasty</td>
<td></td>
</tr>
<tr>
<td>0.31±0.17</td>
<td>0.34±0.16</td>
</tr>
<tr>
<td>0.35±0.10</td>
<td>0.36±0.10</td>
</tr>
<tr>
<td>Immediately after angioplasty</td>
<td></td>
</tr>
<tr>
<td>0.01±0.24‡</td>
<td>0.05±0.24‡</td>
</tr>
<tr>
<td>0.10±0.19‡</td>
<td>0.05±0.15‡</td>
</tr>
<tr>
<td>28 days after angioplasty</td>
<td></td>
</tr>
<tr>
<td>0.46±0.38§</td>
<td>0.49±0.36§</td>
</tr>
<tr>
<td>0.24±0.21§</td>
<td>0.23±0.23§</td>
</tr>
<tr>
<td>ΔStenosis</td>
<td></td>
</tr>
<tr>
<td>0.46±0.38</td>
<td>0.53±0.38</td>
</tr>
<tr>
<td>0.14±0.23</td>
<td>0.18±0.18</td>
</tr>
<tr>
<td>CSA (%)</td>
<td></td>
</tr>
<tr>
<td>48±29</td>
<td>...</td>
</tr>
<tr>
<td>22±16</td>
<td>...</td>
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</tbody>
</table>

Values are mean±1 SD. Heparin, rabbits treated with heparin at time of angioplasty; Hirudin, rabbits treated with recombinant desulphatohirudin at time of angioplasty; NS, not significant; ΔDiameter, difference between luminal diameter measured immediately after angioplasty and diameter measured 28 days after angioplasty; Stenosis (%), percent stenosis expressed as a decimal; ΔStenosis, difference between stenosis immediately after angioplasty and stenosis 28 days after angioplasty (expressed as decimal); CSA, cross-sectional area narrowed by plaque 28 days after angioplasty.

*p≤0.001 vs. within-group luminal diameter before angioplasty; †p≤0.01 vs. within-group luminal diameter immediately after angioplasty; ‡p≤0.001 vs. within-group stenosis before angioplasty; §p≤0.001 vs. within-group stenosis immediately after angioplasty.
Of the 22 femoral arteries of rabbits killed 2 hours after angioplasty, 15 (68%) were narrowed 0–25% in cross-sectional area, and six (27%) were narrowed 26–50%. None of these 22 had luminal narrowing greater than 75%. Planimetric analysis showed similar mean percent luminal narrowing between the heparin-treated and r-hirudin–treated groups (25±20% versus 28±23%, p=0.76).

Of the 26 femoral arteries of rabbits killed 28 days after uncomplicated angioplasty, the number that narrowed more than 50% in cross-sectional area was lower among rabbits treated with r-hirudin than among those treated with heparin. (Six of 14 arteries in the heparin-treated group had a greater than 50% cross-sectional area narrowing of the lumen by plaque, whereas no arteries in the r-hirudin–treated group were narrowed by greater than 50% [p=0.02] [Figure 5].) By blinded planimetric analysis, the mean percent reduction in luminal cross-sectional area was significantly less in the r-hirudin–treated group than in the heparin-treated group (22±16% versus 48±29%, p=0.01) (Table 2 and Figures 6 and 7).

FIGURE 3. A series of angiograms before, after, and 28 days after balloon angioplasty in a heparin-treated rabbit. Panel A: Preangioplasty angiogram demonstrating bilateral focal femoral atherosclerosis (arrows), which is more marked on the left. Panel B: Ten-minute postangioplasty angiogram demonstrating acute angiographic success (arrows) at both sites. Note probable dissection (curved arrow) of left femoral artery. Panel C: Twenty-eight-day angiogram showing bilateral restenosis (arrows). RFA, right femoral artery; LFA, left femoral artery.

FIGURE 4. Plot showing change in luminal diameter (L.D.) between that measured 2 hours after angioplasty and that measured 28 days after angioplasty for arteries with initial success (≥20% increase in luminal diameter) in heparin-treated (n=8) and recombinant desulphatohirudin–treated (n=11) rabbits. Mean decrease in luminal diameter was 0.92±0.61 mm in heparin-treated group and 0.30±0.33 mm in hirudin-treated group.
Luminal thrombus. Thrombi were found at the site of angioplasty at necropsy in three (6%) of the 50 femoral arteries: two were nonocclusive and were found in femoral arteries excised 2 hours after angioplasty (one heparin-treated and one r-hirudin-treated artery), and one was occlusive in a heparin-treated artery excised at 28 days.

Plaque tear. Damage to the arterial wall was apparent in 42 (84%) of the 50 femoral arteries studied. Of these, most tears were into the media (28 [56%]), and the internal elastic lamina was disrupted with its torn edges appearing to have rolled back over its intact portions (Figures 6 and 8). The tears in the plaque extended to the internal elastic lamina in four (8%) and into the adventitia in eight (16%) and appeared to have perforated the wall in two (4%) arteries. Hemorrhage into the plaque, media, or adventitia was found in 25 (50%) arteries (Figure 8). Medial necrosis was moderate or severe in 21 (42%) arteries. Inflammatory cell infiltrates were moderate or extensive in 20 (40%) arteries. Similar numbers of arteries in each group had histological evidence of balloon-induced deep arterial injury with tears into the media (12 of 13 for heparin-treated and 13 of 15 for r-hirudin-treated arteries).

Plaque composition. By planimetric analysis of Movat-stained sections of all arterial segments, the plaques consisted primarily of cellular and acellular fibrous tissue (92±17%). The percent of plaque occupied by foam cells ranged from 0% to 74%, but the mean percent (for all arteries) was only 8±17%. Calcific deposits were not found in any plaque. The percentage of plaque occupied by foam cells was not significantly different between r-hirudin–treated and heparin-treated groups killed 2 hours after angioplasty (0% versus 3±6%, p=0.18). In rabbits killed 28 days after angioplasty, the arteries of those treated with r-hirudin had a lower percentage of plaque occupied by foam cells compared with the arteries of those treated with heparin (2±4% versus 22±25%, p=0.009) (Figures 6 and 7). No differences were found in the area of the media between the four groups.

Discussion

We have shown that rabbits treated with a 2-hour infusion of r-hirudin compared with those receiving bolus heparin had significantly less restenosis 28 days after balloon angioplasty assessed by angiography (luminal diameter reduction) and by quantitative histopathology (luminal cross-sectional area narrowing by plaque at necropsy). This study supports the hypothesis that thrombin is an important mediator of the restenosis process.

The pivotal role of thrombin in platelet aggregation, thrombosis, and hemostasis is well established. More recently, thrombin's stimulatory effect on smooth muscle cell proliferation, its inhibition of endothelial cell growth, its effects on monocytic adhesion, and its modulatory role on vessel wall matrix composition have been reported. These observations suggest that thrombin plays an important role in vessel healing after injury. The advent of specific and potent inhibitors of thrombin has made it possible to investigate the importance of these thrombin effects. In a pig model of carotid artery injury, r-hirudin decreased platelet and fibrinogen deposition and eliminated macroscopic mural thrombus formation in a dose-dependent manner. Our study addressed the role of thrombin inhibition with r-hirudin on neointimal proliferation after balloon angioplasty.

The dose of r-hirudin used in this study was based on previously published work showing that hirudin in a bolus of 1 mg/kg followed by an infusion for 2 hours was significantly more effective than high-dose heparin in preventing thrombus formation after balloon angioplasty in pigs. In a follow-up dose-ranging study, Heras et al showed that 1 mg/kg of r-hirudin was more efficacious than 0.3 or 0.7 mg/kg in preventing mural thrombosis in the normal pig carotid model. The infusion duration was selected based on the observation that maximal platelet deposition occurred during the initial 2 hours after balloon angioplasty in rabbits. The heparin dose of 150 units/kg is similar to the dose at the time of human angioplasty and prolongs the partial thromboplastin time to greater than 1.5 times control.

The mechanism by which a short-term (2-hour) intravenous infusion of hirudin might limit restenosis in our model remains unanswered. In an everted femoral artery model, argatroban, a synthetic competitive thrombin inhibitor, was superior to intravenous or intra-arterial heparin at maintaining vessel patency and attenuating the thrombogenic stimulus for up to 3 hours after the end of the infusion. The mechanism by which a short-term infusion of an
antithrombin agent is able to “pacify” the vessel wall is not known. These studies, however, highlight the pivotal role of thrombin in platelet-rich arterial thrombosis. Our study did not specifically address mural thrombus formation, but thrombosis was infrequent by light microscopy at 2 hours despite a similar number of vessels in each treatment group with deep arterial injury (r-hirudin–treated group, one of eight arteries; heparin-treated group, one of 14 arteries). It has been shown using quantitative histological methods that platelet deposition is more intense when angioplasty is associated with deep and extensive vascular damage. Our finding might be explained by the reduced sensitivity of light microscopy for the detection of platelet thrombi (compared with indium 111–labeled platelet scintigraphy or electron microscopy). An alternative explanation would be that all rabbits killed at 2 hours after angioplasty, whether in the heparin or r-hirudin treatment groups, were effectively anticoagulated with partial thromboplastin times greater than 1.5 times control. It should be noted that balloon angioplasty represents a second injury in our model. A recent publication suggests that platelets and smooth muscle cells react differently to first and second injuries 7 days apart in spite of the fact that both injuries were performed in the same manner. First injury was followed by a monolayer of platelets, but reinjury stimulated extensive

FIGURE 6. Micrographs of histological sections of femoral artery of rabbit treated with heparin and killed 28 days after balloon angioplasty showing total occlusion of lumen by foam cells (FC) and fibrous tissue (FT). Internal elastic lamina (IEL) is disrupted, and torn edges are rolled back over itself. M, media. Movat stains; magnification, ×55 for top panel and ×160 for bottom panel.
thrombosis. The authors suggest that injured neointimal smooth muscle cells may represent an important stimulus to platelet reactions, with mural thrombosis more common after reinjury. This may prove to be an important factor in the selection and dosing of antithrombotic agents after balloon angioplasty.

We also noted a significantly smaller mean percent of the plaque area occupied by foam cells in the r-hirudin–treated arteries at 28 days after angioplasty compared with that in the heparin-treated arteries (2±4% versus 22±25%, p=0.009). Although the explanation for this observation is unknown, it might be speculated that it is mediated through an antithrombin effect. Thrombin has been shown to promote monocyte adhesion to the vessel wall. The inhibition of this effect by r-hirudin may in part explain r-hirudin's antiproliferative effect.

The suitability of animal models of experimental atherosclerosis and balloon angioplasty in addressing restenosis after human coronary angioplasty has been questioned. However, the lesions seen in this model have similar morphological characteristics to those seen in human coronary arteries. The atherosclerotic plaques in this study were predominantly fibrocellular in composition (92±17%). This high percentage of fibrous tissue is consistent with that found in studies of the composition of atherosclerotic plaques in the coronary arteries of patients with fatal...
Figure 8. Micrographs of sections of femoral arteries of rabbits treated with heparin and killed 2 hours after balloon angioplasty showing tearing of the atherosclerotic plaque (1), internal elastic lamina (2), and media (3) associated with hemorrhage (4). L, lumen; P, plaque; M, media. Movat stain; magnification, ×125 for panel A and ×80 for panel B.

Acute myocardial infarction and sudden coronary death. Moreover, the small percentage (8±17%) of plaque area occupied by foam cells using air desiccation endothelial injury and cholesterol feeding is similar to that found in the coronary arteries of patients under 40 years of age with fatal coronary artery disease (Dollar A.L. et al, manuscript submitted), further supporting the suitability of this rabbit model for these studies. In this study, the angiographic percent stenosis was approximately 30% before balloon angioplasty and corresponded to an angiographic luminal diameter of 1.16–1.24 mm. Despite potential improvements in quantitation of luminal diameter and morphometric analysis, these vessel dimensions and morphological features are similar to our previous publications and underscore the consistency of this animal model.

In conclusion, this study supports the hypothesis that thrombin plays an important role in the restenosis process after balloon angioplasty. We have demonstrated that specific inhibition of thrombin by r-hirudin at the time of angioplasty significantly reduces angiographic restenosis and cross-sectional area luminal narrowing by quantitative histopathology.
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