Specific Factor Xa Inhibition Reduces Restenosis After Balloon Angioplasty of Atherosclerotic Femoral Arteries in Rabbits

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Background Balloon angioplasty of atherosclerotic arteries results in activation of the coagulation cascade. Several coagulation factors, including factor Xa and thrombin, are mitogenic for vascular smooth muscle cells in vitro and thus may play a role in restenosis after balloon angioplasty. Specific inhibition of factor Xa can be achieved with recombinant antistasin (rATS) or tick anticoagulant peptide (rTAP). We hypothesized that inhibition of Xa would limit restenosis after balloon angioplasty in an atherosclerotic rabbit model.

Methods and Results Focal femoral atherosclerosis was induced by air desiccation injury and a high-cholesterol diet in 38 New Zealand White rabbits. Recombinant antistasin (n=20 arteries) or rTAP (n=14 arteries) was administered by intravenous bolus at the time of balloon angioplasty and followed by a 2-hour infusion; controls (n=21 arteries) received bolus heparin alone (150 U/kg). Therapeutic prolongation of the activated partial thromboplastin time occurred, and antithrombotic drug levels were achieved in all animals. Luminal diameter in millimeters by quantitative angiography did not differ between treatment groups before (1.1±0.2 for controls, 1.1±0.2 for rATS, and 1.1±0.3 for rTAP) or after balloon angioplasty (1.5±0.3 for controls, 1.4±0.2 for rATS, and 1.4±0.2 for rTAP). At 28 days, treatment with factor Xa inhibitors tended to result in arteries with larger luminal diameter than controls (1.2±0.3 for rATS, 1.2±0.3 for rTAP versus 1.0±0.3 for control, P=.09 by one-way ANOVA). Restenosis, defined as reduction in angiographic luminal diameter (in mm) from 2 hours after angioplasty to 28 days after angioplasty was less in the rATS group than in controls (−0.2±0.1 versus −0.5±0.4, P<.001) and tended to be less in the rTAP group (−0.3±0.2 versus −0.5±0.4, P=.07). Quantitative histopathological analysis showed less percent cross-sectional area narrowing by plaque in both rATS- and rTAP-treated arteries compared with controls (42±21%, 47±18%, and 63±14%, respectively; P<.01 by one-way ANOVA).

Conclusions We conclude that a 2-hour infusion of rATS or rTAP reduced angiographic restenosis and resulted in less luminal cross-sectional narrowing by plaque compared with controls. (Circulation. 1994;89:1262-1271.)

Key Words • thrombosis • antistasin • peptides • coagulation

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Despite more than a decade of intense research, the effectiveness of coronary balloon angioplasty remains limited by a 30% to 50% restenosis rate within 6 months of successful dilatation.1-3 Fibromuscular intimal hyperplasia, the pathophysiological substrate of restenosis in the majority of patients, is believed to result from a complex series of biological processes initiated by arterial injury and followed by platelet deposition and mural thrombosis with the subsequent release of chemotactic and mitogenic factors. These factors may initiate smooth muscle cell migration and/or proliferation in the intima, followed by elaboration and deposition of extracellular matrix and ultimately resulting in intimal plaque growth.1,2,4-6

Arterial injury is known to activate the coagulation cascade through the intrinsic or extrinsic pathways with the generation of factor Xa. Factor Xa in combination with the nonenzymatic cofactor Va and calcium assembles into a prothrombinase complex on the surface membrane of activated platelets and catalyzes the conversion of prothrombin to thrombin. Factors X, Xa, protein S, and thrombin have recently been shown to stimulate smooth muscle cell proliferation in vitro.7,10

We have demonstrated that specific inhibition of thrombin by recombinant desulfatohirudin reduces restenosis after balloon angioplasty in a rabbit model of focal femoral atherosclerosis.11

Recently, two highly selective polypeptide inhibitors of factor Xa have been described.12-18 Antistasin is a 119-amino-acid polypeptide originally isolated from the salivary glands of the Mexican leech Haementeria officinalis. Tick anticoagulant peptide is a 60-amino-acid protein first purified from extracts of the soft tick Ornithodoros moubata. Both proteins have been produced as recombinant gene products and characterized as competitive, tight-binding, reversible inhibitors of factor Xa with subnanomolar dissociation constants.14,18

Recombinant antistasin (rATS) and recombinant tick
anticoagulant peptide (rTAP) are highly selective for factor Xa and have no effects on platelets or other serine proteases, including thrombin. The antithrombotic efficacy of rATS and rTAP has been previously demonstrated in a rabbit model of thromboplastin-induced venous thrombosis and several animal models of acute arterial thrombosis.

Because the procoagulant and mitogenic effects of factor Xa may play a role in restenosis after balloon angioplasty, we hypothesized that inhibition of factor Xa would reduce restenosis after balloon angioplasty in a rabbit model of focal femoral atherosclerosis.

**Methods**

**Induction of Focal Femoral Atherosclerosis**

Thirty-eight male New Zealand White rabbits (4.0±0.4 kg) were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Focal atherosclerosis was induced in 1- to 2-cm femoral artery segments by air desiccation endothelial injury followed by a 2% cholesterol–6% peanut oil diet for 28 days as described previously (Fig 1). This diet has been shown to increase cholesterol levels 20- to 25-fold. The rabbits were housed according to Animal Welfare Act specifications, and all surgical procedures were performed using sterile technique and general anesthesia.

**Drug Administration**

Animals were enrolled consecutively beginning with control animals, followed by the rATS-treated group, followed by the rTAP group. Control animals (n=19) received a single bolus of heparin (150 U/kg) (heparin sodium injection, 1000 USP U/mL, porcine intestinal mucosa, Solopak Laboratories) to prevent clot formation on the catheters. No additional heparin was administered after the bolus dose in these control animals. Purified rATS and rTAP were obtained from Merck Sharp and Dohme Research Laboratories. The preparation of these proteins has been described previously. Eleven animals received rATS intravenously as a 0.5-mg/kg bolus followed by infusion of 0.25 mg·kg⁻¹·h⁻¹ for 1 hour and 0.125 mg·kg⁻¹·h⁻¹ for a second hour. Eight rabbits received rTAP intravenously as a 1.0-mg/kg bolus followed by infusion of 2.2 mg·kg⁻¹·h⁻¹ for 1 hour and 1.1 mg·kg⁻¹·h⁻¹ for an additional hour. The 5- to 10-fold difference between the doses of rATS and rTAP used in this study was predicted to be equipotent with respect to factor Xa inhibition based on the results from previous studies in which the antithrombotic efficacies of both inhibitors were evaluated and compared in a venous model of thrombosis.

**Balloon Angioplasty**

Induction of focal femoral atherosclerosis resulted in complete occlusion in 2 of the 16 femoral arteries in animals selected for rTAP treatment, in 2 of the 22 arteries in animals selected for rATS treatment, and in none of the 38 femoral arteries in control animals. Seventeen atherosclerotic arteries in control animals were patent but not ballooned as part of this protocol. Thus, balloon angioplasty was performed 28 days after endothelial injury and cholesterol feeding in 21 femoral arteries of control rabbits, in 20 arteries of rabbits receiving rATS, and in 14 arteries of animals treated with rTAP. After right carotid arteriotomy, a 5F Berman catheter (Arrow International, Inc) was advanced into the descending aorta and positioned two vertebral spaces above the aortic bifurcation. Baseline angiography of the iliac and femoral arteries was performed using a Siemens Optilux Angiographic System (model 1179878VS048, Siemens Aktiengesellschaft), and im-
ages were recorded on 35-mm cineradiographic film using 5 mL Hypaque 76 (diatrizoate meglumine and diatrizoate sodium injection USP, Winthrop-Breon Laboratories) diluted with 5 mL of sterile saline. A grid with 1-cm markings was used as an internal calibration standard. The angiographic catheter was replaced by a 0.014-in. guide wire, and a 2.5-mm ACS balloon dilatation catheter (Advanced Cardiovascular Systems, Inc) was centered across the femoral stenosis under fluoroscopic guidance. Three 60-second, 10-atm balloon inflations were performed at 1-minute intervals using a hand inflater (Advanced Cardiovascular Systems, Inc). After balloon angioplasty of the contratral femoral stenosis with the same protocol, the balloon catheter was removed and replaced by the 5F Berman catheter, and angiography was repeated after 10 minutes to ensure arterial patency and after 2 hours for quantitative angiographic analysis. The catheter and vascular sheath were then withdrawn, the carotid artery was ligated, and the wound was closed. Rabbits were kept alive for 28 days after balloon angioplasty and fed normal rabbit chow. Final angiography was performed just before they were killed, using the left carotid artery as described above.

**Blood Samples**

Activated partial thromboplastin time (aPTT) and plasma levels of rATS and rTAP were determined before drug administration, after angioplasty, and at the conclusion of the 2-hour drug infusion. Plasma levels of rATS and rTAP were measured as described previously.\(^{19}\)

**Pressure Perfusion and Specimen Preparation**

After the 28-day angiogram, the animals were killed with an overdose of Nembutal, and the distal aorta and iliac arteries were perfused at physiological pressure (100 mm Hg and 22°C) with 100 mL of 10% buffered formaldehyde. The angioplastied femoral artery segments (approximately 4 to 5 cm) were excised bilaterally with the proximal and distal ends marked with silk sutures. The specimens were postfixed in 10% buffered formaldehyde and prepared for light microscopy.

**Angiography**

All 2-hour postangioplasty angiograms were analyzed quantitatively using a computer-assisted technique described previously.\(^{11,25}\) The minimum luminal diameters (mm) at the site of focal femoral artery stenoses and segments proximal and distal to the stenoses were determined with the aid of a computer-generated line placed perpendicular to the long axis of the artery. The percent stenosis was derived from the ratio of the minimal luminal diameter to the average of the proximal and distal segments. Templates were drawn of the preangioplasty angiogram to ensure that analysis was performed on the same arterial segments.

**Histopathology**

Each excised femoral arterial segment was cut in cross section at 1- to 2-mm intervals, dehydrated in ethanol and xylene, and embedded in paraffin. Sections from each 1- to 2-mm segment were stained by hematoxylin and eosin and by the Movat method.\(^{26}\) Quantitative histopathology was performed by an experienced vascular pathologist blinded to the treatment groups. For quantitative comparisons, the segment with the greatest luminal cross-sectional area narrowing was identified for each angioplasty site. Luminal narrowing was determined by computerized planimetry using a CUE-2 image analyzer (Galai Productions, Ltd) in association with an Olympus BH-2 microprojection system as described previously.\(^{27}\) Sections were also categorized with respect to extent of plaque tear (to internal elastic lamina, into the media, into the adventitia, or complete perforation), the presence of luminal thrombi, hemorrhage into the plaque, extent of medial necrosis, and extent of inflammatory cell infiltrates. Computer-assisted planimetric assessment of plaque composition in-cluded determination of the areas of plaque occupied by fibrous tissue (cellular and acellular) and foam cells.

**Statistical Analysis**

Data are reported as the number of femoral arteries in each experimental group and expressed as the mean±SD. Angiographic and histopathological differences between treatment groups at a given time point were analyzed using one-way ANOVA followed by unpaired Student’s t test to evaluate two-tailed levels of significance. Paired tests were used for intragroup angiographic comparisons. For values that were not normally distributed, a Mann-Whitney U test was used. Comparison of categorical data was made using the two-tailed Fisher’s exact test. A value of P≤.025 was considered significant to account for the Bonferroni correction when comparing the two treatment groups with controls. Otherwise, P≤.05 was considered significant.

Sample size calculations were made as follows. We assumed that the change in minimal luminal diameter from immediately before the balloon angioplasty to 28 days after balloon angioplasty (the primary end point in this restenosis study) would be at least −0.5±0.4.\(^{11}\) Because of limited drug availability, the study was designed to use 50% more control than treatment experiments. To have a probability of >.80 of finding a significant treatment effect (in terms of angiographic diameter change from after angioplasty to 28 days) if the true difference between groups was 0.25 mm (50% reduction in angiographic restenosis), we would require sample sizes of 20 for controls, 13 for rATS, and 13 for rTAP. This is based on a two-sided hypothesis at the P=.05 level.

**Results**

**aPTTs and Drug Levels**

The mean aPTT at baseline for all animals was 129±25 seconds. Ten minutes after drug bolus and the completion of angioplasty, the aPTT was >200 seconds in all control and rATS-treated animals and in five of six (83%) of the rTAP-treated animals. Two hours after the heparin bolus, the aPTT remained >200 seconds in the controls. Two hours after drug bolus and at the end of the 2-hour drug infusion, the aPTT was >200 seconds in the rATS-treated animals but was >200 seconds at 2 hours in only five of eight (63%) of the rTAP-treated animals.

The mean rATS plasma level concentration was undetectable at baseline, increased to 183±25 nmol/L after drug bolus, and then decreased modestly to a concentration of 147±26 nmol/L at the end of the 2-hour infusion. The rTAP-treated animals achieved plasma inhibotor concentrations approximately sixfold to sevenfold higher, yielding plasma rTAP levels of 1145±190 nmol/L after drug bolus and 1054±239 nmol/L at the end of the 2-hour infusion. These drug levels were similar to those shown to be fully antithrombotic in a recent study using a rabbit model of venous thrombosis and similar drug levels correlated with an aPTT of 1.5 to 3.0 times control.\(^{19}\)

**Angiographic Data**

The angiographic data are summarized in Table 1. The mean minimal luminal diameter of the normal femoral artery segment proximal to the stenosis was 1.9±0.2 mm. Fig 2 depicts the individual minimal luminal diameter before, 2 hours after, and 28 days after angioplasty in each of the three treatment groups. The mean minimal luminal diameters increased significantly after balloon angioplasty in all three groups (P=.0001) without signif-
TABLE 1. Luminal Diameters and Percent Stenosis Before, 2 Hours After, and 28 Days After Angioplasty

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before</th>
<th>After</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=21)</td>
<td>1.1±0.2</td>
<td>1.5±0.3*</td>
<td>1.0±0.3†</td>
</tr>
<tr>
<td>rATS (n=20)</td>
<td>1.1±0.2</td>
<td>1.4±0.2*</td>
<td>1.2±0.3†</td>
</tr>
<tr>
<td>rTAP (n=14)</td>
<td>1.1±0.3</td>
<td>1.4±0.2*</td>
<td>1.2±0.3†</td>
</tr>
</tbody>
</table>

Stenosis, %

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>rATS</th>
<th>rTAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>36±12</td>
<td>39±12</td>
<td>40±14</td>
</tr>
<tr>
<td>After</td>
<td>11±19†</td>
<td>17±14‡</td>
<td>14±17‡</td>
</tr>
<tr>
<td>28 Days</td>
<td>39±19§</td>
<td>28±16§</td>
<td>33±15§</td>
</tr>
</tbody>
</table>

Control indicates rabbits treated with heparin at time of angioplasty; rATS, rabbits treated with recombinant antistasin at time of angioplasty; rTAP, rabbits treated with recombinant tick anticoagulant peptide at time of angioplasty; n, number of arteries in each treatment group; and stenosis, percent stenosis. Values are mean±1 SD.

*P<.001 vs luminal diameter before angioplasty (paired t test).
†P<.001 vs luminal diameter immediately after angioplasty (paired t test).
‡P<.001 vs percent stenosis before angioplasty (paired t test).
§P<.001 vs percent stenosis immediately after angioplasty (paired t test).

By one-way ANOVA, P=.09 for luminal diameter at 28 days and P=.09 for percent stenosis at 28 days.

ificant differences between groups either before or after angioplasty (Table 1). By 28 days after balloon angioplasty, the mean minimal luminal diameter decreased significantly in each of the three groups. Animals treated with factor Xa inhibition tended to have larger luminal diameters at 28 days than controls (1.2±0.3 mm for rATS-treated arteries and 1.2±0.3 mm for rTAP-treated arteries versus 1.0±0.3 mm for controls, P=.09 by one-way ANOVA). When the mean minimal preangioplasty dimension for each group was compared with the 28-day luminal diameter, only the rATS-treated arteries maintained a larger luminal diameter (1.1±0.2 versus 1.2±0.3 mm, P=.0002).

Restenosis, the primary angiographic end point of the study and defined as the change in luminal diameter (in mm) from 2 hours after angioplasty to 28 days after angioplasty, was significantly less in the arteries of animals receiving factor Xa inhibition (P=.002 by one-way ANOVA) with the greatest effect seen in animals treated with rATS (−0.2±0.1 versus −0.5±0.4 mm, P<.001) (Fig 3A). There was a trend toward less restenosis when comparing arteries receiving rTAP with controls (−0.3±0.2 versus −0.5±0.4 mm, P=.07).

When minimal luminal dimensions are expressed as percent stenosis, the findings are similar. The percent stenosis 28 days after balloon angioplasty in animals treated with factor Xa inhibition tended to be less than for controls (28±16% for rATS-treated arteries and 33±15% for rTAP-treated arteries versus 39±19% for controls, P=.09 by one-way ANOVA). The change in mean percent stenosis from after angioplasty to 28 days, an important angiographic parameter of restenosis, was less in the groups treated with factor Xa inhibition than in controls (P=.06 by one-way ANOVA). The change in mean percent stenosis for rATS-treated animals was significantly less than for controls (11±7% versus 28±24%, P=.0001) (Fig 3B). The change in mean percent stenosis from immediately after angioplasty to 28 days in the rTAP group tended to be less than for controls, although this was not statistically significant (16±14% versus 28±24%, P=.18).

Among the 21 control arteries, one artery was occluded at 28 days, and one artery showed a disproportionate increase in luminal diameter after balloon angioplasty. When these two “outliers” are excluded, the angiographic results are similar. There was no difference in minimal luminal diameter either before angioplasty (1.1±0.2 versus 1.1±0.2 versus 1.1±0.3 mm for controls, rATS-treated arteries, and rTAP-treated arteries, respectively; P=NS), after angioplasty (1.5±0.2 versus 1.4±0.2 versus 1.4±0.2 mm, P=NS), or 28 days after angioplasty (1.1±0.2 versus 1.2±0.3 versus 1.2±0.3 mm, P=NS). The change in luminal diameter (in mm), however, was different among the groups (−0.4±0.2 versus −0.2±0.1 versus −0.3±0.3, P<.01).

Histopathology

Quantitative histopathology was performed on 19 control, 19 rATS-treated arteries, and 13 rTAP-treated arteries. The remaining four arteries could not be analyzed for technical reasons (Table 2).

Fig 2. Plots of minimal luminal diameter (in mm) before balloon angioplasty (BA), 2 hours after BA, and 28 days after BA for 21 control arteries, 20 antistasin-treated arteries, and 14 tick anticoagulant peptide–treated arteries.
FIG 3. Scatterplots of change in luminal diameter (in mm; A) and change in percent stenosis (B) from 2 hours after angioplasty to 28 days after angioplasty for each of the three treatment groups. TAP indicates tick anticoagulant peptide. (P=.002 for change in luminal diameter and P=.006 for change in percent stenosis by one-way ANOVA.)

*Luminal Narrowing*

Computerized planimetry showed significantly less mean percent luminal cross-sectional area narrowing by plaque in arteries from animals treated with factor Xa inhibition than in controls (P=.002 by one-way ANOVA). Both rATS- and rTAP-treated animals showed less percent cross-sectional area narrowing by plaque than controls (42±21% for rATS, 47±18% for rTAP, and 63±14% for controls, P=.001 control versus rATS and P=.01 control versus rTAP) (Fig 4). When the two outlying control arteries described above were excluded, the results are similar (60±12% for controls versus 42±21% for rATS-treated arteries versus 47±18% for rTAP-treated arteries, P<.01).

*Extent of Vascular Injury*

There was histological evidence of plaque tear at sites of angioplasty in 15 of 19 arteries receiving rATS, in all 19 control arteries, and in all 13 receiving rTAP.

**TABLE 2. Histopathology 28 Days After Balloon Angioplasty**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=19)</th>
<th>rATS (n=19)</th>
<th>rTAP (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSA narrowing by plaque, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All arteries*</td>
<td>63±14</td>
<td>42±21†</td>
<td>47±18†</td>
</tr>
<tr>
<td>Arteries with plaque tear (n)</td>
<td>63±14 (19)</td>
<td>48±19† (15)</td>
<td>47±18† (13)</td>
</tr>
<tr>
<td>Extent of arterial injury (no. of arteries)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque tear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to IEL</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Into media</td>
<td>9</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>To adventitia</td>
<td>10</td>
<td>3‡</td>
<td>3</td>
</tr>
<tr>
<td>Perforation</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>15‡</td>
<td>13</td>
</tr>
<tr>
<td>Severe medial necrosis</td>
<td>18</td>
<td>10†</td>
<td>6†</td>
</tr>
<tr>
<td>Inflammation</td>
<td>18</td>
<td>6†</td>
<td>4†</td>
</tr>
<tr>
<td>Plaque composition, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrocellular</td>
<td>91±12</td>
<td>96±7</td>
<td>94±6</td>
</tr>
<tr>
<td>Foam cells</td>
<td>9±12</td>
<td>4±7</td>
<td>6±6</td>
</tr>
<tr>
<td>Calcium</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Control, rabbits treated with heparin at time of angioplasty; rATS, rabbits treated with recombinant antistasin at time of angioplasty; rTAP, rabbits treated with recombinant tick anticoagulant peptide at time of angioplasty; CSA, cross-sectional area; and IEL, internal elastic lamina. Values are mean±1 SE.

*P=.002 by one-way ANOVA.
†P=.01 vs control arteries.
‡P=.05 vs control arteries.
Importance of Early Events After Angioplasty in Restenosis

Pathological studies of arteries after balloon angioplasty reveal endothelial denudation, tearing of plaque, and dissection into the media with hemorrhage and necrosis. As a consequence of this extensive injury, platelets are deposited and mural thrombosis occurs. In experimental models of balloon angioplasty, platelet deposition and thrombus formation peak within 24 hours of injury.28,29 In a rabbit model, the proliferation of smooth muscle cells also begins early, reaching a peak 72 hours after injury.30 These data suggest that cellular responses to balloon injury occur early. This argument is supported by the present study in which a 2-hour infusion of specific factor Xa inhibitors with potent antithrombotic effects administered at the time of balloon angioplasty reduced restenosis measured 28 days later.

Role of Factor Xa and Thrombin in Restenosis

Factor Xa and thrombin may be important mediators of restenosis by promoting mural thrombosis, by direct mitogenic effects, or by stimulating the release of growth factors. Several experimental models have documented mural thrombus at the site of balloon dilation in the early hours after balloon angioplasty, and the extent of mural thrombosis correlates with the extent of deep (medial) injury.28,31-35 Mural thrombus has also been documented in the coronary arteries of humans after balloon angioplasty.36,37 Acute ischemic syndromes known to be associated with mural thrombosis (unstable angina or acute myocardial infarction) are associated with increased restenosis risk.3,38 The incorporation of mural thrombus into an atherosclerotic plaque has been proposed as a mechanism of the rapid progression of atherosclerosis.39 A similar mechanism may be important in restenosis. In addition, factors X and Xa and thrombin are potent mitogens of both cultured fibroblasts and smooth muscle cells in vitro.7-10 Thrombin has been shown to stimulate the release of a PDGF-like substance from cultured endothelial cells, suggesting that thrombin-induced mitogenesis may be mediated through one or more cellular growth factors.40,41 We have previously shown that specific thrombin inhibition with recombinant desulfatohirudin significantly limits restenosis in our rabbit model, suggesting a critical role of thrombin in this complex biological process.11 Thus, factor Xa may influence restenosis directly or indirectly through effects of thrombin or other growth factors or mitogens.

Factor Xa is a serine protease that, along with calcium, phospholipid, and factor Va, catalyzes the conversion of prothrombin to thrombin via the prothrombinase complex. This complex amplifies the conversion of prothrombin to thrombin by 280,000-fold,31 and small amounts of thrombin can accelerate this process through a feedback mechanism. Targeting the prothrombinase complex through factor Xa inhibition thus represents a logical antithrombotic approach.

Failure of Conventional Anticoagulants to Impact on Restenosis

If thrombosis plays an important role in restenosis, why have conventional anticoagulants like heparin...
failed to prevent human restenosis. This is not surprising because even high doses of heparin are ineffective at preventing thrombosis. Thrombin bound by fibrin clot remains enzymatically active and is resistant to inactivation by the heparin-antithrombin III complex. Bar-Shavit and colleagues showed that thrombin can bind to subendothelial extracellular matrix, remain enzymatically active, and be spared inactivation by circulating inhibitors. The inadequacy of heparin at preventing thrombus is further supported in experiments with a pig carotid model of angioplasty. In these studies, doses of heparin up to 250 U·kg⁻¹·h⁻¹ failed to prevent mural thrombosis after balloon angioplasty, whereas hirudin, a potent antithrombin III-independent thrombin inhibitor, was highly effective in preventing mural thrombosis in the rabbit. Similar data are not available for factor Xa inhibitors, but these agents have been shown to be highly effective in preventing thrombus formation in a venous thrombosis model and models of acute arterial thrombosis. Thus, preventing thrombin generation through the inhibition of factor Xa may be a more effective method of preventing mural thrombus formation after arterial injury than heparin. Agents such as factor Xa inhibitors and antithrombins may replace current anticoagulants such as heparin used during and after balloon angioplasty for both preventing mural thrombus formation and limiting restenosis.

**Differences in Factor Xa Inhibitors**

Both rATS and rTAP are potent and highly selective factor Xa inhibitors. Neither has direct effects on platelets or other proteins in the coagulation cascade. Importantly, these agents have no direct antithrombin effect. Recombinant antistasin and rTAP differ mainly in their duration of action, with rATS having a longer half-life because of its slower clearance. The plasma levels achieved with both inhibitors in this study were comparable to those shown to be fully antithrombotic in a previous study of venous thrombosis in rabbits. Furthermore, the more pronounced prolongation of the aPTT by rATS compared with rTAP, even at lower plasma concentrations, is consistent with results obtained with these inhibitors in a variety of animal thrombosis models. This property has been shown to reflect a kinetic difference in the rate of factor Xa inactivation in the prothrombinase complex by these two inhibitors. Although we have not determined the clearance rate of either factor Xa inhibitor in our study, results from a previous study suggest a slow clearance rate for rATS in rabbits as evidenced by the slow rate of depletion of plasma rATS levels as well as the sluggish
return of aPTT values toward baseline after termination of the rATS infusion. In contrast, the rTAP plasma levels measured in the same study dropped rapidly during the 60-minute period after termination of the rTAP infusion. Preliminary results obtained from studies using rhesus monkeys yield values for the circulating half-life of rTAP and rATS of 60 minutes and 10 hours, respectively, confirming the dramatically different clearance rates for these inhibitors (C.T. Dunwoodie and G.P. Vlasuk, unpublished data).

In our study, rATS reduced angiographic restenosis and luminal cross-sectional narrowing by plaque, whereas rTAP did not significantly reduce the angiographic parameters of restenosis, although a clear trend was evident. Sample size calculations were based on the assumption that rTAP would reduce the change in luminal diameter by at least 50% compared with controls. We observed a lesser reduction (40%), and the statistical power for this result is only 50%. Limited drug quantities precluded additional experiments to increase the sample size, so the angiographic results for rTAP are not conclusive. However, rTAP significantly reduced luminal narrowing by histopathology, although the effect was less pronounced than with rATS. This difference between these factor Xa inhibitors may be the result of the shorter duration of action of rTAP. The length of time that an antithrombin or antithrombotic effect requires to prevent restenosis is not known. Because factor Xa and thrombin may play pivotal roles early, the different durations of action of these two factor Xa inhibitors may explain the observed difference in restenosis rates between the two agents. It is possible that a higher dose of rTAP or a prolonged infusion would have resulted in more significant anticoagulation and improved efficacy.

Study Limitations

The applicability of animal models to human percutaneous transluminal coronary angioplasty is uncertain. The hypercholesterolemic rabbit iliac artery model using balloon injury to initiate the atherosclerotic process has been criticized as not representative of human atherosclerosis because of the high percentage of foam cells in the plaque. Our rabbit model differs in that endothelial injury is initiated by air desiccation injury rather than by balloon endothelial denudation. Air desiccation injury and cholesterol feeding result in a fibrocellular plaque with few foam cells (approximately 2% to 4%), which is similar in composition to the atherosclerotic plaques of young patients who die suddenly of acute myocardial infarction. In addition, this model is a “double-injury” model in which an atherosclerotic artery, rather than a normal artery, undergoes balloon angioplasty. The resulting arterial injury and repair processes may thus be more akin to human balloon angioplasty.

In this study, both femoral arteries were used (if available), and each vessel was treated as an independent unit of observation. We believe this is justified because analysis of the data on an animal-by-animal basis, performed by averaging the values for paired arteries within the same animal, yielded similar results (change in luminal diameter [in mm]: controls, −0.4±0.2 [n=18]; rATS-treated animals, −0.2±0.1 [n=11], and rTAP-treated animals, −0.3±0.2 [n=8], P=.02 by one-way ANOVA; percent cross-sectional area narrowing by plaque: controls, 60±12%; rATS-treated animals, 41±19%; rTAP-treated animals, 49±15%; F=0.01 by one-way ANOVA).

Because of limited drug availability and logistical details of the animal model, rabbits were enrolled in a consecutive fashion by a single experienced operator. To ensure that no bias was introduced before drug treatment, angiography was performed before and after angioplasty. No differences with respect to any important angiographic parameters were detected between the treatment groups.

Mural thrombus was seen in only one control artery at 28 days. This finding does not exclude thrombus formation as a major component in postangioplasty restenosis. We have previously reported that mural thrombus occurs in 50% of arteries examined 24 hours after bolus heparin treatment and balloon angioplasty. At 28 days, however, identification of thrombus and differentiation from atherosclerotic plaque are problematic. Several studies in human coronary arteries have shown a decline in the frequency of mural thrombus at necropsy related to the estimated time of thrombosis before death. It may be that mural thrombi spontaneously lyse by 28 days or are incorporated into the atherosclerotic vascular wall and are not apparent 28 days after angioplasty using standard histological techniques. Assessing the degree of arterial injury 28 days after angioplasty is problematic for many reasons. Intimal proliferation, organization of mural thrombus, and migration of cellular elements can obscure the extent of initial arterial injury. Although we have reported the depth of apparent injury at 28 days, we cannot be certain that these data represent the true initial injury resulting from the fully standardized balloon angioplasty protocol.

Conclusions

In summary, potent and specific inhibition of factor Xa with rATS or rTAP at the time of balloon angioplasty resulted in less restenosis by quantitative angiography and less luminal cross-sectional narrowing by plaque at 28 days compared with controls. This study complements our previous work in the same animal model using the specific thrombin inhibitor recombinant desulfatothirudin and lends support to the hypothesis that elements of the coagulation system, either through thrombus formation or because of other mitogenic effects, are important in restenosis after balloon angioplasty in this animal model.

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