Prolonged Thrombin Inhibition Reduces Restenosis After Balloon Angioplasty in Porcine Coronary Arteries

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Background—Arterial injury after percutaneous transluminal coronary angioplasty (PTCA) triggers acute thrombus formation and thrombin generation. Hirudin, a potent and direct thrombin inhibitor, prevents thrombus formation after arterial injury. Two large clinical trials showed marked reduction in acute clinical events but no long-term benefits in reducing restenosis during short-term administration of thrombin inhibitors. Our hypothesis is that adequate, maintained thrombin inhibition, by inhibiting all the thrombin-dependent mechanisms, will reduce neointima formation after PTCA.

Methods and Results—Thirty-six pigs received three different regimens of hirudin: bolus (1 mg/kg), short-term (bolus +0.7 mg/kg per day for 2 days), and long-term (bolus +0.7 mg/kg per day for 14 days). The results on neointima formation at 4 weeks after coronary angioplasty were compared with the control group (100 IU heparin/kg bolus). Hirudin was continuously administered for 2 weeks through an infusion pump. In vivo thrombin generation was persistently increased up to 2 weeks after angioplasty. Inhibition of thrombin activity for 14 days reduced luminal narrowing by 40% (58 ± 3% versus 35 ± 3%; P < .001). No differences were observed among the bolus and short-term hirudin groups and the control group.

Conclusions—Our results indicate that there is a continued, marked thrombin generation that lasts for at least 2 weeks after PTCA. Administration of r-hirudin for 2 weeks significantly reduces neointima formation after PTCA. This observation, if extrapolated to humans, could explain the lack of effect on restenosis observed in the clinical trials with antithrombin agents despite the clear benefits on reducing acute thrombotic complications after PTCA. Therefore an adequate and prolonged administration of thrombin inhibitors is needed to “passivate” the thrombogenic substrate (disrupted arterial wall) and achieve full benefit of this therapeutic approach. (Circulation. 1998;97:581-588.)

Key Words: restenosis • thrombin inhibition • angioplasty

Despite its widespread use, the effectiveness of coronary balloon angioplasty is limited by the high rate of restenosis. Attempts to modify the restenotic process have yielded uniformly disappointing results. Repeated failure to reduce the incidence of restenosis reflects a basic lack of understanding of the pathogenesis of the vascular response to injury. Balloon angioplasty causes severe vascular injury, as evidenced by the significant fracture of the atherosclerotic plaque. Similarly, there are areas of endothelial denudation, intimal disruption, and necrosis of smooth muscle cells. Restenosis is a reparative process that is activated in response to injury induced by balloon angioplasty. Mural thrombus formation occurs immediately after injury and is followed by smooth muscle cell activation, migration, proliferation, and increased synthesis of extracellular matrix that tends to cease within the first 3 months.1,2

Thrombin is generated in large amounts at the site of injury after balloon angioplasty and is amplified by formation of the prothrombinase complex both in humans and animals.3–5 Exposure of tissue factor in the atherosclerotic plaque to flowing blood leads to increased thrombin generation, resulting in platelet- and fibrin-rich thrombus formation.6–7 The important role of thrombin in generating acute platelet-rich thrombus and vascular healing after arterial injury is well documented.8–11 Thrombin is also the most potent activator of platelets, and its expression is upregulated in smooth muscle cells after severe arterial injury. Thrombin also mediates several cellular activities involved in the restenosis process, including smooth muscle cell migration and proliferation.12

Hirudin is a potent, specific, direct thrombin inhibitor. It is highly effective at preventing acute platelet-rich thrombosis after deep arterial injury.9–11 r-Hirudin reduced restenosis after balloon angioplasty in atherosclerotic femoral arteries in rabbits.13 The results obtained in minipigs after short-term administration of r-hirudin are controversial; one group reported significant inhibition of neointimal thickening after carotid angioplasty,14 whereas another report found no effect on neointimal formation.15
However, two large clinical trials showed acute reduction in clinical events but no long-term benefit in reducing restenosis during short-term administration of hirudin (HELVETICA trial) and hirulog (Hirulog Angioplasty Study). In addition, combined analysis of the results of GUSTO IIb and TIMI 9b trials suggests a modest but significant reduction of 13% (P=0.026) in the incidence of (re)infarction with hirudin versus heparin in patients with an acute coronary syndrome (Reference 18 and data presented at the 1996 AHA Scientific Sessions). Likewise, GUSTO IIb showed a significant 62% reduction in death or myocardial infarction (P<0.001) after 24 hours of hirudin administration but only a mild effect at 30 days (10% reduction; P<0.058). Thus short-term hirudin does not appear to protect against long-term processes of thrombosis and proliferation that involves thrombin activation and arterial plaque disruption.

We hypothesized that to achieve the full benefit of direct thrombin inhibition, an adequate and prolonged administration of thrombin inhibitor is required to passivate the injured vascular wall. Therefore this study was designed to test whether effective and maintained inhibition of thrombin activity for 2 weeks would reduce long-term luminal narrowing after coronary balloon angioplasty.

Methods

Animal Species

This study was performed with Yorkshire Albino pigs (weight, 27 to 32 kg). All animals were purchased from a single local farm. Intervention procedures and animal handling were approved by the Mount Sinai School of Medicine animal management program, which is accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). The AAALAC meets NIH standards as set forth in the “Guide for the Care and Use of Laboratory Animals” (DHHS Publication No. [NIH] 85 to 23, Revised 1985) according to the PHS “Policy on Humane Care and Use of Laboratory Animals for Awardee Institutions” and the AHA “Guidelines for the Use of Animals in Research.”

Recombinant Hirudin

Hirudin is a potent anticoagulant derived from the European leech Hirudo medicinalis. Hirudin is a single polypeptide of 65 amino acids that binds by its carboxy terminus to the substrate recognition site of thrombin and by its amino terminus to the catalytic center of thrombin. In the present study, we used recombinant desulfatohirudin (r-hirudin; GCP-39393; Revasc, Ciba-Geigy Corp), which is identical to its natural version except for a missing sulfate group on the tyrosine-63.

Experimental Design

The experiment consisted of four groups: a control group (n=10 animals; 22 coronary arteries) treated with a single intravenous bolus of heparin given 15 minutes before the balloon angioplasty and three different groups being treated with different regimens of r-hirudin–treated animals. The regimens of hirudin administration were as follows.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>Hirudin-Bolus Group</td>
<td>This group (n=9 animals; 17 coronary arteries) received an intravenous bolus (1 mg/kg) given 15 minutes before the balloon angioplasty.</td>
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<tr>
<td>Short-term Hirudin Group</td>
<td>This group (n=8 animals; 12 coronary arteries) received an intravenous bolus (1 mg/kg) given 15 minutes before the balloon angioplasty followed by a continuous intravenous infusion of 0.7 mg hirudin/kg per hour maintained for 2 days through an implantable infusion pump. The administration of hirudin for 2 days was selected to mimic the design of the human studies in which the mean length of administration of hirudin was 2.1 days.</td>
</tr>
<tr>
<td>Long-term Hirudin Group</td>
<td>This group (n=9 animals; 15 coronary arteries) received an intravenous bolus (1 mg/kg) 15 minutes before the balloon angioplasty followed by a continuous intravenous infusion of 0.7 mg hirudin/kg per hour maintained for 14 days through an implantable infusion pump. The 2-week time period was selected on the basis of our previous studies indicating that the thrombogenicity of an injured artery, evaluated as deposition of indium-labeled platelets, lasts for up to 11 days after carotid angioplasty in the pig. The effects of the hirudin administration on neointima formation after coronary angioplasty were evaluated at 4 weeks after the coronary intervention and compared with the heparin-treated group that serves as control.</td>
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Installation of Infusion Pump

Pigs were premedicated with ketamine (15 mg/kg IM) and then deeply anesthetized with pentobarbital (25 mg/kg), intubated, and mechanically ventilated with room air. Continuous monitoring of the ECG and arterial pressure was performed. The right carotid artery and internal jugular vein were exposed after a medial incision on the anterior surface of the neck. Both vessels were cannulated with separate 0.8-mm Teflon fluorinated ethylene polypropylene tubing. After the vessels were cannulated, the opposite end of each tube was tunneled subcutaneously, exiting on the dorsal side of the neck. The carotid cannula was used as a blood sampling port. The venous cannula was secured to a Synchronized 8615 miniature infusion pump with an internal 18-mL reservoir (Medtronic Inc). The pump was secured to the animals with a specially designed vest. On the 2nd day (short-term) or 14th day (long-term) after balloon angioplasty, the pump was removed and both arterial and venous cannulas were sealed.

Coronary Balloon Angioplasty

After the infusion pump was completely installed and homeostasis ensured, a 4-cm-long 8F introducer sheath was placed into the right carotid artery. At this point, the animals serving as controls were anticoagulated with heparin, and hirudin was administered to the three experimental groups. An appropriate PTCA catheter (8F) was inserted through the right carotid artery and advanced under fluoroscopic guidance (Phillips BV-24) into the left and right coronary arteries for angioplasty of the left anterior descending coronary artery, left circumflex artery, and right coronary artery, respectively. Coronary angioplasty was performed by three inflations at 10 atm of a 3.0-mm angioplasty balloon (Cordis Corp). This angioplasty procedure resulted in overstretching of the artery, causing severe arterial injury. Angiographic examinations were performed before, during, and after PTCA, and x-ray films were taken at each intervention. After the angioplasty procedure, all animals were allowed to recover and returned to the pens until their predetermined time for euthanasia. All animals were fed a normal diet (Purina Farm Chow) with free access to water and were allowed to move freely within their pens.

Blood Sampling and Hematological Parameters

Blood samples were drawn through the internal jugular cannula immediately before anticoagulation, 15 minutes after anticoagulation, and immediately after balloon angioplasty. Subsequent blood sampling was performed on days 2, 6, 10, 14, and 28 after balloon angioplasty. aPTT times were determined by timed assays with a Diagnostica Stago 7 System.
Determination of Plasma Levels of Thrombin-Hirudin Complex

None of the commercially available assays for the determination of thrombin generation cross-react with the porcine model. Plasma levels of THC as a molecular probe for thrombin generation in vitro and in vivo has been previously validated in the pig. An ELISA was developed for the determination of THC in porcine plasma. The assay is a modification of the one developed by Bichler et al. The assay involves the use of a monoclonal antibody to the porcine THC that binds selectively to the thrombin component of the THC. Antithrombin antiserum was filtered through a prothrombin-Sepharose column, and the unbound fraction was further purified by affinity chromatography on porcine thrombin-Sepharose. Antihirudin immunoglobulin G (IgG) from sheep (kindly provided by R. Maschler, GEN, Munich, Germany) was purified by affinity chromatography as described. The ELISA plates (Immunolon F) were coated with rabbit anti-porcine thrombin-hirudin IgG. The solid-phase antithrombin antibody binds selectively to the thrombin component of the THC. Hirudin bound to thrombin is recognized by a sheep anti-hirudin IgG and detected by rabbit anti-sheep peroxidase conjugate. The absorbance is then at 405 nm and compared with the standard curve. The detection limit of the thrombin-hirudin ELISA is 600 pg of THC/mL of plasma. This assay for thrombin generation is based on levels of THC formed by the 1:1 stoichiometric binding of hirudin:thrombin.

Because of the requirement of hirudin for the determination of THC, we were only able to study thrombin generation during the 2-week period of hirudin administration. However, in humans, directional changes of prothrombin fragment F1.2 and THC were similar to the ones reported here.

To assure that the dosage of hirudin could bind all the thrombin being generated, we measured the plasma levels of free hirudin at different time points after PTCA. Measurements were achieved by use of a specific ELISA (American Stago). The plasma levels of free hirudin achieved by its continuous administration was an average of 2500 ng/mL blood. This level of hirudin was in excess to neutralize all the thrombin being generated as a consequence of the coronary angioplasty and was not a limiting factor for total thrombin inhibition.

Fixation, Harvesting, and Pathological Examination of Injured Vessels

Animals were euthanatized on day 28 after PTCA. At that time, the animals were again deeply anesthetized with ketamine and pentobarbital and fully heparinized (100 units/kg IV). The aorta and heart were exposed through a median thoracotomy, and the animals were euthanatized by overdoses of pentobarbital. The heart and ascending aorta were immediately excised, and a large-bore catheter was inserted retrogradely into the ascending aorta. The coronary arteries were flushed with 1 L of physiological buffer (0.1 mol/L PBS containing heparin and papaverine, pH 7.4) followed by perfusion fixation with 1 L cold (4°C) 4% paraformaldehyde in 0.1 mol/L PBS, pH 7.4. All coronary perfusions were performed at 100 mm Hg. After perfusion fixation, the heart was placed in 1 L of fresh fixative and allowed to stand overnight at 4°C. The coronary segments of interest were fixed, dehydrated in ethanol and xylene, embedded in paraffin at 59°C, sectioned (4 μm), and stained by combined Masson-elastin method. Histological sections were analyzed as described below.

Histomorphometric Analysis

All 2-mm coronary segments were analyzed by two experienced observers blinded to the treatment group. For each coronary artery that underwent angioplasty, the section with the most extensive injury-induced response was identified and evaluated. The degree of arterial injury induced during the angioplasty was classified according to an injury score, modified from Schwartz et al, defined as follows: 0, endothelium intact, no injury; 1, endothelium denuded, internal elastic lamina intact; 2, internal elastic lamina lacerated, media exposed but not lacerated; 3, internal elastic lamina lacerated, media visibly lacerated but external elastic lamina intact; and 4, external elastic lamina lacerated, large laceration of media extending through the external elastic lamina.

Only those segments with an injury score ≥3 were retained for further analysis. The segment most narrowed by plaque (ie, that section with the narrowest lumen) was then further evaluated by computer-assisted histomorphometry. Photomicrographs were captured on Adobe Photoshop 6.0 and transformed to NIH Image 1.6 for quantitative analysis. All procedures were performed on a Power Macintosh 8100/100.

The following parameters were measured: LA=luminal area, area of arterial lumen; MA=medial area, original medial layer encircled by the internal elastic lamina (IEL) and external elastic lamina (EEL); VA=vessel area, total arterial area encircled by the EEL; OM=length of the outer media; MT=medial thickness (average of five measurements); and IA=intimal area, area occupied by the neointima.

The neointima was further subdivided into three distinct areas that were clearly visible on direct histological examination. These three areas were defined as residual thrombus, submedial hematoma, and fibrocellular hyperplasia (see Fig 1). Residual thrombus was defined as thrombus or remnants of an original thrombus induced at the time of angioplasty and that was undergoing organization. Thrombus under a medial flap or dissection was referred to as a hematoma. Fibrocellular hyperplasia was defined as those areas comprised solely of cellular elements and extracellular matrix, as seen on microscopic analysis of stained tissues.

From these measurements the following parameter was calculated

% Luminal Narrowing=100×IA/(LA+IA)

To ensure that potential differences between groups were due to treatment and not to differences in the severity of arterial injury, a damage index score and percentage of missing EEL score was determined for every section studied. The damage index was calculated as the ratio between the total vessel area (VA), and the area of the circle formed by the length of outer media measured directly, assuming that there is no loss of medial tissue. The percentage of missing EEL was calculated as the difference between the IEL of a circle derived from the measured EEL and medial thickness, and the IEL measured directly by computerized analysis.

Damage Index=OM2/4π
VA

% Missing Internal Elastic Lamina (MIEL)=
(EEL−2πMT−IEL)
(EEL−2πMT)×100

Figure 1. Photomicrograph of a typical coronary section 4 weeks after PTCA, indicating the three distinct processes that contribute to neointima thickening. IEL indicates internal elastic lamina; EEL, external elastic lamina.
Statistical Analysis
Data are presented as number of coronary segments in each experimental group and expressed as mean±SEM. Areas are expressed as mm². The statistical significance of differences between the normal and treated groups was determined with a two-way ANOVA followed by an unpaired Student’s t test to evaluate two-tailed levels of significance, and multiple regression analysis was used for the evaluation of variable dependence. Differences were considered significant at P<.05.

Results
Arterial Injury Induced by the Angioplasty Procedure
All coronary segments analyzed for intimal proliferation after angioplasty were histologically characterized by disruption of the IEL, with laceration of the tunica media and exposure of the EEL. As shown in the Table, the degree of arterial injury induced by the coronary intervention was similar for all the groups.

Hematological Parameters
Activated Partial Thromboplastin Time
The aPTT ratio for the control group (heparin bolus) was 2.3±0.2 and 1.9±0.3 immediately before and after PTCA, respectively. In all the hirudin-treated groups, the administration of the bolus induced a similar prolongation of the aPTT ratio (2.1±0.2) as the one induced by heparin in the control group. The coronary intervention was associated with a reduction in the prolongation of the aPTT value after the administration of bolus of hirudin (1.64±0.2 versus 2.1±0.2 after the bolus). The aPTT ratios achieved at the time of PTCA and at different time points during the 14 days of continuous administration in the long-term hirudin-treated animals are shown in Fig 2. Even though the initial aim of the study was to maintain an aPTT ratio among 2 and 3 times control, as seen in Fig 2 the antithrombin regimen used attained slightly higher aPTT ratios. No bleeding complications were observed in any of the animals for the duration of the study.

Thrombin-Hirudin Complex
Plasma levels of THC were determined and served as a marker for thrombin generation. The corresponding values of THC in the long-term hirudin group are reported in Fig 3. A significant increase in plasma levels of THC was observed immediately after balloon angioplasty. This observation clearly indicates that the arterial injury inflicted by the coronary intervention is associated with a surge in thrombin generation.

Increased thrombin generation persisted for at least 14 days after balloon angioplasty. Given that the longest hirudin administration was stopped on the 14th day after intervention, determination of THC was not possible thereafter.

Luminal Stenosis After Arterial Injury
When the animals were euthanatized (4 weeks after angioplasty), all groups showed similar coronary artery sizes, assessed by the vascular area encircled by the EEL. The analysis of the percentage of lumen narrowing indicated a statistically significant larger lumen in the 2-week hirudin-treated group com-

Table: Degree of Injury

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Damage Index (x±SEM)</th>
<th>% Missing IEL (x±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>0.32±0.02</td>
<td>30.0±3.6</td>
</tr>
<tr>
<td>Hirudin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bolus</td>
<td>17</td>
<td>0.24±0.07</td>
<td>24.6±3.0</td>
</tr>
<tr>
<td>2 days</td>
<td>12</td>
<td>0.25±0.06</td>
<td>25.1±2.1</td>
</tr>
<tr>
<td>2 weeks</td>
<td>15</td>
<td>0.27±0.04</td>
<td>26.2±2.5</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
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*IEL indicates internal elastic lamina.

Figure 2. Values of aPTTs achieved at the time of PTCA and at different time points during the administration of the thrombin inhibitor r-hirudin. Results expressed as aPTT ratio vs. baseline.

Figure 3. Plasma levels of THC at different time points during the administration of r-hirudin. Results are expressed as pg/mL of blood. The results clearly indicate that PTCA is associated with a significant, maintained thrombin generation that persists for at least 14 days after balloon angioplasty.
pared with the other groups \((P > 0.01)\). No differences were observed among the heparin-control group, the bolus-hirudin, and 2-day hirudin groups (Fig 4). These results indicate that the significant reduction observed in the luminal area after angioplasty is due to the development of neointima rather than to remodeling or vascular shrinkage because the vessel area, as defined by the area encircled by the EEL, was similar in all groups.

**Histomorphometry**

Representative photomicrographs of histological sections from the control and 2-week hirudin-treated groups are shown in Fig 5. These figures show a significant intimal thickening in the coronary arteries of the control animals. The newly formed proliferative tissue, characterized by spindle-shaped cells, filled medial tears extending between adjacent medial areas and encroaching into the lumen. On immunohistochemistry, the majority of these cells stained positive for \(\alpha\)-actin, indicating their smooth muscle cell origin (data not shown). Fig 6 depicts the values of the three major contributors (residual thrombus, submedial hematoma, and fibrocellular hyperplasia) to luminal narrowing in these two groups. Bolus and short-term administration of hirudin did not induce any differences compared with the heparin control group.

Overall, the inhibition of thrombin activity for 14 days after PTCA by hirudin resulted in a 40% reduction in luminal

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**Figure 4.** Percent of luminal narrowing at 4 weeks after balloon angioplasty for the control and the three hirudin-treated groups. (\(x \pm \text{SEM}; * P < 0.01\)).

**Figure 5.** Representative photomicrographs of combined Masson-elastin–stained histological sections from control (left) and 2-week hirudin-treated animals (right).
narrowing in comparison to the control group (from 58.3 ± 3.5% to 35.1 ± 3.2%, respectively; \( P < .005 \)) (Fig 6A). The inhibition of thrombin activity achieved by the 2-week continuous infusion of hirudin induced a significant reduction (\( P < .01 \)) in the contribution of residual thrombus to luminal narrowing from 10.9 ± 2.1% in the control animals to 4.2 ± 1.0% (Fig 6B). The contribution of submedial hematoma to luminal narrowing was also reduced by the long-term antithrombin treatment from 6.2 ± 1.4% in the control animals to 4.1 ± 1.1% in the treated animals (\( P < .05 \)) (Fig 6C). There was also a significant reduction in the fibrocellular component of the neointima from 41.3 ± 2.3% to 26.8 ± 3.1%, respectively (\( P < .01 \)) (Fig 6D).

**Discussion**

This study shows that effective and sustained inhibition of thrombin activity after PTCA significantly reduces the formation of intimal thickening after PTCA. Our observations strongly support the hypothesis that thrombin plays an important role in mediating the restenosis process. Furthermore, these results emphasize the importance of the dosage and duration of administration of hirudin in achieving optimal and adequate antithrombin therapy.

The earliest event after balloon angioplasty in the pig is the appearance of a platelet- and fibrin-rich thrombus at the site of injury.\(^{129,130,131,132,133,134,135} \) The mural thrombus may act as a growth factor–rich structure with the release of the platelet α-granule contents during platelet aggregation and thrombus formation. Platelets contain platelet-derived growth factor and transforming growth factor-β, among other growth and mitogenic factors. In addition, the presence of a mural thrombus may also contribute to late neointimal thickening and restenosis. This process may involve the organization of the thrombotic mass and release of growth and mitogenic factors and cytokines. These factors in turn stimulate smooth muscle cell migration and proliferation, which leads to intimal hyperplasia and extracellular matrix production. Thus it is possible that initial thrombus formation correlates with later neointimal thickening.

The important role of thrombin as a major agonist for platelet activation and thrombosis is well established. More recently, thrombin has also been identified as a growth-promoting factor for smooth muscle cells, and its modulator role in vessel wall matrix composition has been reported.\(^{12,28,29} \) With the advent of specific and potent direct thrombin inhibitors such as hirudin, the thrombin–mediated effects on restenosis after PTCA can be investigated.

The importance of thrombin inhibition for reducing acute thrombotic complications early after severe arterial injury was well illustrated by two clinical trials using r-hirudin and one of its analogues, hirulog, as antithrombin probes. Thrombin inhibition significantly reduced the incidence of acute cardiac events during active drug administration. However, no impact on late restenosis was observed.\(^{16,17} \) Hirudin is a specific and direct inhibitor of thrombin activity, not thrombin generation. Therefore, soon after the infusion ceases, no further effect could be expected if thrombin is still being generated. The marked and continued generation of thrombin for at least 2 weeks after balloon angioplasty in our model illustrates the importance of duration of treatment. Therefore, insufficient duration of administration (2 to 3 days in the longest human trial), inadequate route of administration (subcutaneous versus intravenous) to maintain adequate blood levels, or both may explain the lack of positive results for prevention of late restenosis in these clinical studies.

We previously showed that thrombin inhibition with hirudin reduces acute fibrin deposition at lower doses (aPTT, 1.7 times control) in a porcine model of angioplasty. When higher doses (aPTT > 2 times control) were used, acute mural
thrombosis was totally abolished and platelet–vessel wall inter-
an action was reduced to a monolayer.9,10

Further evidence supporting the hypothesis of thrombin as a
major mediator of restenosis comes from studies using hirudin11
and recombinant tick anticoagulant peptide (rTAP) in the cho-
lesterol-fed rabbit femoral artery model.12 TAP, by binding and
inhibiting factor Xa, also inhibits thrombin generation at the
initiation of the coagulation cascade. In the first study, hirudin was
administered to rabbits for only 2 hours after balloon angioplasty.

The authors found a trend toward reduced cross-sectional nar-
rowing. The effectiveness of a prolonged inhibition of antithrom-
botic activity was initially suggested by Schwartz et al13 in the pig
model after metallic stent implantation. The authors demonstrated
a decrease in neointimal thickening in coronary arteries after
administration of TAP for 5 days.

The importance of the experimental model for studying
therapeutic interventions to reduce restenosis is a critical factor.
It is clearly emphasized by a recent study comparing the effects
of thrombin inhibition by hirudin in three animal models (rats,
rabbits, and minipigs). This study showed that short-term
hirudin administration reduced neointima formation in the
rabbit model, but no inhibition of neointimal growth was seen
after short-term administration in rats and minipigs.14

The most often-used animal models to study experimental
restenosis after arterial injury are rats, rabbits, and pigs.15–18
Among the different animal models available to study neo-
intima thickening after arterial injury, the pig coronary model
appears to be the most relevant to human coronary restenosis.
The porcine model shares similar cardiovascular anatomy and
physiology and therefore may provide results more predictive
of responses in humans.19–24 On this basis, the animal model
selected for the present study was balloon angioplasty of the pig
coronary arteries.

In our study we addressed the effects of direct and specific
inhibition of thrombin activity by using r-hirudin on neo-
intimal formation after coronary angioplasty in the pig. The
periods of antithrombin treatment were selected to cover (a)
the acute thrombogenicity (bolus), (b) short term (2 days) to
mimic the human studies, and (c) long term (2 weeks) on the
basis of a previous study indicating that the thrombogenicity of
an injured artery lasts up to 11 days after PTCA.25

Thrombin is the most potent activator of platelets in
humans. Hirudin has the greatest binding affinity of any
antithrombin agent (kD 2×10-10 for recombinant hirudin).
The dose of hirudin used in this study was based on our
previous studies showing that a 1 mg/kg bolus followed by a
2-hour infusion prevented thrombus formation after balloon
angioplasty.9,10

The oversized balloon injury model used in this study
creates a reproducible and reliable arterial damage that is similar
to that observed in humans. The degree of arterial injury
induced at the time of angioplasty is a major predictor of the
neointimal response.41,42 Our experimental model of coronary
angioplasty causes deep arterial injury and medial tears with
exposure of the external elastic lamina. As shown in the Table,
the degree of injury did not differ between coronary arteries
taken from the two groups. Therefore, the observed differ-
ces in neointima formation cannot be attributed to different
levels of injury.

Our data support the hypothesis that acute thrombus formation
after angioplasty contributes to the deposition of new mass in the
vessel wall and to luminal narrowing. By direct histological
analysis of the stained coronary segments, we showed that
organizing thrombus, submedial hematoma, and fibrocellular
hyperplasia are all present 4 weeks after angioplasty. The presence
of these elements contributes to the overall luminal narrowing.
Thrombus may also provide a matrix for cellular elements to
invade and proliferate. In addition, it constitutes a reservoir for
active substances such as platelet-derived growth factors, throm-
bin, and fibrinogen, which have all been shown to influence
cellular proliferation.43–45 Therefore, the observed inhibitory effect
of r-hirudin on restenosis might be exerted via two different
mechanisms. First, by directly reducing the overall size of acute
thrombus, and indirectly through an inhibitory effect of cell
proliferation as a consequence of a reduction in the release of
growth factors originating during the process of thrombus forma-
tion (PDGF, etc).

Additional support for the efficacy of adequate antithrom-
botic therapy for prevention of restenosis may also be extrap-
olated from the clinical results of the recent EPIC and
EPILOR trials.46,47 These studies suggest that the inhibition of
acute thrombotic complications after PTCA by use of the
specific antagonist of the IIb/IIIa platelet receptor complex,
Rheo-Pro, is associated with a reduced rate of “clinical
restenosis.” Such observations are based on clinical symptom-
atology, since angiography was not included in the initial
design of the study.

Our results in the porcine model indicate that there is
continued and marked generation of thrombin for at least 2
weeks after balloon angioplasty. This finding suggests that after
balloon injury thrombin generation continues for a longer
period of time than initially thought. If this observation is
extrapolated to humans, it could explain the lack of effect on
restenosis observed in clinical trials with antithrombin agents
developed despite their significant effect on reducing acute thrombotic
complications of PTCA. Therefore the effects of an effective
and prolonged inhibition of thrombin on restenosis after
PTCA in humans deserve to be studied.

In conclusion, our results clearly indicate that to achieve the
full benefits of the therapeutic treatment with direct thrombin
inhibitors, an appropriate dosage and prolonged administration
of these agents is needed to “passivate” the thrombogenic
substrate (injured arterial wall) and achieve the full benefits of
this therapeutic approach.

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maintenance of the animals.

References
1. Fuster V, Falk E, Fallon JT, Badimon L, Chesebro JH, Badimon JJ. The
three processes leading to post PTCA restenosis: dependent on the lesion
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