Effects of Thrombin Inhibition on the Development of Acute Platelet-Thrombus Deposition During Angioplasty in Pigs
Heparin Versus Recombinant Hirudin, a Specific Thrombin Inhibitor

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The effect of recombinant hirudin and the dosage of heparin on acute platelet-thrombus deposition during carotid angioplasty in anesthetized pigs was prospectively assessed. Fifty-five animals (mean weight, 33.9 kg) were randomized to one of six heparin dosages: heparin boluses of 35, 50, 100, 150, 200, or 250 units/kg followed by a continuous infusion of 35, 50, 100, 150, 200, or 250 units/kg/hr, respectively. Another five pigs received a bolus of 1 mg/kg hirudin (recombinant desulphatohirudin), a specific thrombin inhibitor, followed by an infusion of 1 mg/kg/hr. Bilateral carotid angioplasty was performed in all pigs 20 minutes after starting the infusion; they were sacrificed 57±12 minutes after the procedure. Deep medial arterial injury was present in approximately 75% of the dilated segments, and subendothelial injury in the remaining 25%. Mean log of number of platelets and molecules of fibrinogen per centimeter squared of deep injury in segments from all the animals treated with heparin were 4.74±1.03 and 5.02±0.64, respectively. A regression analysis showed an inverse correlation of the log of platelet deposition with the heparin group (r = -0.56, p = 0.0001) with administered total units of heparin (r = -0.55, p = 0.0003) and with mean plasma heparin concentration (r = -0.55, p = 0.0004) in deeply injured arteries. Similar inverse relations were obtained for fibrinogen. In contrast, the deposition of platelets and fibrinogen in subendothelial injury was very low and independent of the heparin administered. Macroscopic thrombus decreased from 72% of deeply injured arterial segments in the lowest heparin dosage group (activated partial thromboplastin time similar to the animals in the hirudin group) to 10% in the highest heparin dosage group and to 0% in the animals treated with hirudin. Activated partial thromboplastin times were threefold that of control basal values for animals on the lowest heparin dosage (35 units/kg); a similar prolongation of times was observed in the hirudin group. However, the mean logs of platelet and fibrinogen deposition per centimeter squared of deep injury were significantly lower in the hirudin group than on 35 units/kg heparin (3.23±0.44 vs. 5.44±0.60 and 3.98±0.36 vs. 5.33±0.53, respectively; p < 0.001) and were also lower in the hirudin group than on the highest dosage (250 units/kg) of heparin (3.8±0.6 and 4.6±0.6, respectively; p < 0.04). In the presence of deep but not subendothelial arterial injury after angioplasty, there is a dose-dependent inhibition by heparin of acute platelet and fibrinogen depositions and the proportion of arterial segments with mural thrombosis. Hirudin was more effective in preventing thrombosis than heparin; this probably reflects the more specific and potent inhibition of thrombin by hirudin. (Circulation 1989;79:657–665)
Acute thrombotic coronary occlusion is a major setback after an otherwise successful angioplasty and occurs in 2–12% of patients. Platelets and thrombus deposited at the site of deep arterial disruption appear to have an important role in restenosis.\(^1\)\(^2\) The arterial injury exposes collagen in the media or the plaque and releases thromboplastin; both activate the coagulation system and result in a major generation of thrombin that is amplified 280,000 times by the prothrombinase complex.\(^3\) Thrombin is a potent stimulus for platelet activation and results in a positive feedback for activation of factors V and VIII, conversion of fibrinogen to fibrin, and cross-linking of fibrin by activation of factor XIII.\(^4\) Heparin is a coenzyme of circulating antithrombin III (AT III) and greatly accelerates the interaction of AT III with thrombin. In a recent retrospective study, heparin reduced platelet deposition and acute thrombosis during experimental angioplasty.\(^5\) In the present study, our aim was to extend this observation and to determine the effect of increasing heparin dosages and antithrombin activity on platelet and fibrinogen deposition and macroscopic mural thrombosis after deep arterial injury. We hypothesized that mural thrombosis and deposition of platelets and fibrinogen would decrease as dosages and concentrations of heparin and its antithrombin activity increased. To elucidate a major mechanism of action of heparin, we also compared heparin with recombinant desulphathirudin, a potent and highly specific thrombin inhibitor.

**Methods**

We studied 60 normal pigs, of the Babcoek four-way cross stock (mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) (43 males and 17 females; 4 months old; mean weight, 33.9±3 kg; range, 24–44 kg) that were obtained from local farmers, housed at the Mayo Institute Hills Farm, Rochester, Minnesota, and fed a normal chow diet. Autologous platelets in all pigs were labeled with 300–400 \(\mu\)Ci \(^{111}\)In-tropolone; 250 \(\mu\)Ci of \(^{125}\)I human fibrinogen was administered 24 hours before the procedure. We used labeled human fibrinogen because we found that pig platelets were able to recognize human fibrinogen as ligand. Washed pig platelets were isolated as previously described\(^6\)\(^7\) and resuspended in Tyrode’s buffer with albumin. The washed platelets were stimulated with ADP 10 \(\mu\)M in the presence and absence of human fibrinogen (40–200 \(\mu\)g/ml) and the response measured by impedance platelet aggregation.\(^8\) Pig platelets aggregated in the presence of human fibrinogen but were unable to respond to ADP in its absence. The platelet response in the presence of human fibrinogen was dose dependent from 50 to 200 \(\mu\)g/ml fibrinogen.

**Experimental Protocols**

All pigs were sedated with 300 mg ketamine i.m. (Ketaset, Bristol Laboratories, Syracuse, New York). After inhalation of halothane (Fluothane, Wyeth-Ayerst Laboratories, Philadelphia, Pennsylvania) the pigs were intubated and mechanically ventilated with room air (Harvard respirator, Harvard apparatus, South Natick, Massachusetts) mixed with 0.5% halothane to maintain anesthesia. Although halothane has been reported to decrease in vitro platelet aggregation, we have not yet detected any decrease in platelet deposition in vivo in areas of deep arterial injury comparing placebo-treated pigs anesthetized with halothane with others that received an intravenous mixture of ketamine, fentanyl, and etomidate (unpublished results). The electrocardiogram and intra-arterial pressure were continuously monitored throughout the procedure. Pigs were randomly allocated to one of the following six heparin dosages: heparin boluses of 35, 50, 100, 150, 200, or 250 units/kg followed by a continuous infusion of 35, 50, 100, 150, 200, or 250 units/kg/hr of heparin, respectively. An additional five pigs received the specific thrombin inhibitor, recombinant hirudin (1 mg/kg bolus plus 1 mg/kg/hr infusion). Before heparin or hirudin administration, both femoral arteries and left femoral vein were catheterized. Via the left femoral artery, a No. 8 Medi-tech polyethylene balloon 8 mm × 3 cm (Meditech, Watertown, Massachusetts) was advanced under fluoroscopic guidance into the left common carotid artery for the angioplasty procedure. Via the right femoral artery, an 8F heparin bonded multipurpose catheter (USCI, Billerica, Massachusetts) was placed in the descending aorta to obtain blood samples for heparin concentration and activated partial thromboplastin time (APTT). Heparin and hirudin were infused in the left femoral vein through a No. 15 Angiocath (Deseret, Deseret Medical, Parke-Davis, Sandy, Utah). All the lines were constantly flushed with 5% dextrose. When all the catheters were in place, the bolus of heparin or hirudin was administered followed immediately by the infusion. The infusions (in 0.9% saline) were delivered with a Harvard Pump (Harvard apparatus, South Natick, Massachusetts) at a rate of 0.8 ml/min. Heparin used in this study was heparin sodium derived from porcine intestine that contained 1,000 USP units/ml; all the vials used belonged to the same lot and were purchased from Elkins-Sinn, Cherry Hill, New Jersey. The recombinant hirudin was desulphathirudin from yeast (CGP 39393) with a specific activity of 11,496 ATU/mg.

**Carotid Angioplasty**

Angioplasty of the common carotid arteries was performed with an 8F Medi-tech polyethylene balloon (8 mm × 3 cm) dilatation catheter. Right and left common carotid arterial segments between the second and fourth cervical vertebrae were dilated by
five 30-second inflations to 6 atm (Medi-tech pressure manometer) with 60-second intervals between inflations. Spot films were also taken during balloon inflation (without contrast injection) and immediately before and after the dilatations with 6 ml meglumine diatrizoate (Renografin, Mallinckrodt, St. Louis, Missouri).

**Histopathology**

Before sacrifice, 120 ml of 1% Evans blue dye in 0.9% saline was injected into the ascending aorta. The pigs were given an overdose of pentobarbital and perfused antegradeley with 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M cacodylate (pH 7.25) at a pressure of 100 mm Hg for 15 minutes to allow fixation of the arteries in situ. The carotids were removed and cleaned of all adventitia. The dilated portion of the fixed arteries were easily identified by the Evans blue staining, by the ridges of vasoconstriction proximally and distally to it, and from the spot films taken during and after the angioplasty. The dilated portion of the fixed carotid artery was divided into two equal segments; and a similar-sized segment was taken from the adjacent, proximal, and distal ends. All deep arterial injury was confined to the two segments in the dilated region except in five arteries where a tear extended distally or proximally; in these animals, an extra segment was analyzed. Segments were not analyzable if there was a mechanical problem (slippage of balloon during angioplasty because area of injury was not clearly defined or because of rupture of the artery during angioplasty). Two to three cross-sections of each segment were taken for light microscopy after isotope counting and after planimetry measurement of the area of deep injury. A twofold magnifying lens (Sunnex Laboratories, Needham, Massachusetts) was used to examine for the presence of macroscopic mural thrombus because these may embolize, enlarge, or contribute to more severe vasoconstriction. We previously demonstrated that macroscopic mural thrombosis is associated with at least $20 \times 10^6$ platelets/cm$^2$.

**Quantitative Deposition of Platelet and Fibrinogen**

The extent of platelet deposition on the arterial segments was quantitated by the method of Dewanjee et al with autologous $^{111}$In-labeled platelets. Three blood samples were obtained at the time of death for the determination of mean radioactivity in counts per minute (cpm)/total weight of blood (microbalance), which was corrected for unbound $^{111}$In. Radioactivity (cpm) in each arterial segment was counted with a gamma well counter (Gamma 8000, Beckman Instruments, Fullerton, California) with the spectrometer adjusted to include the photo peaks at 174, 247, and 421 kev (sum peak) of $^{111}$In radionuclide. The $^{111}$In counts per minute per gram of whole blood were converted (cpm/ml); using the whole blood platelet count, the number of platelets per count per minute (plts/cpm) was determined. This $^{111}$In count per minute for each segment of the tissue is multiplied by the platelets per count per minute to determine the platelet deposition that is adjusted for the area.

After decay of $^{111}$In-radionuclide (14 days), tissues and blood were counted for $^{125}$I with the spectrometer window adjusted to include the 28-kev radiograph, the 35-kev gamma ray, and the sum peak at 63 kev. From the plasma fibrinogen levels, the total area of arterial damage, $^{125}$I radioactivity in plasma and arterial segments, and the molecules of fibrinogen per area of damage were calculated. The counts per minute per gram of $^{125}$I radioactivity in plasma was determined as the $^{111}$In counts per minute per gram in whole blood and was corrected for free $^{125}$I unbound in plasma. The whole blood fibrinogen level (mg/dl) was divided by the plasma cpm/gm to yield the number of milligrams of fibrinogen per count per minute. The fibrinogen deposition for each segment is calculated by multiplying the $^{125}$I counts per minute for each segment by milligrams of fibrinogen per count per minute and adjusting for the area. Finally, micrograms of fibrinogen are converted to molecules by multiplying by a constant ($1.77 \times 10^{12}$).

**Planimetry of Damage**

Planimetric measurement of deep injury was performed after measurement of the radioactivity of each arterial segment and before sectioning for histologic examination. Each arterial segment was cut open with a dissecting scissors and pinned onto a paraffin block with a metric grid. To be photographed, the paraffin block is immersed in 0.9% saline in a petri dish to prevent tissue damage from the high-intensity photographic lights.

Each segment is photographed at the highest magnification possible ($\times1.25–5$) using a Tessovar Photomacrophographic Zoom System (Zeiss, Thornwood, New York) under a tungsten light source. The color 3.5x5-in. photographs are labeled, and the region of deep injury (confirmed histologically) outlined with a black marking pen.

Each photograph was measured by planimetry on a Tandy microcomputer interfaced to a digitizing tablet (Houston Hipad, Austin, Texas). The computer is calibrated from the metric grid in each photograph. Two regions are measured for each arterial segment: the total segment area and the areas of deep injury.

From each arterial segment, two to three ring sections were stained with hematoxylin and eosin and Heidenhain's Weigert-Van Gieson stain. Light microscopy by two observers documented the presence of a dissection or tear into the media at the site of dilatation. A tear extending through the internal elastic lamina into the media was defined as deep arterial injury; endothelial denudation without a
tear through the internal elastic lamina was considered subendothelial injury.

**Laboratory Tests**

Blood was drawn from a heparin-bonded catheter (8F) in the abdominal aorta to determine the basal platelet count, hematocrit, fibrinogen, APTT, AT III, and heparin levels. APTT and heparin levels were also determined 10 minutes after starting the heparin infusion, just before the left carotid angioplasty, before the right carotid angioplasty, and immediately before sacrifice. At that moment platelet counts, fibrinogen, hematocrit, and AT III were also determined.

Blood for heparin levels was mixed 9:1 with a 3.85% citrate solution. The samples were inverted to mix and then centrifuged to obtain platelet-poor plasma (PPP). The heparin levels were determined within 6 hours of collection using a commercially available kit (American Scientific Products, McGraw Park, Illinois). The synthetic peptide substrate used in this kit when cleaved by the thrombin remaining in the sample released a fluorescent molecule [5-aminoisophthalic acid, dimethyl ester (AIE)], which was measured using a Protopath® fluorometer.16

An aliquot of PPP was stored on ice or frozen at −20 °C (for up to 30 days) for AT III levels. The AT III levels were determined with a kit purchased from American Scientific Products. The synthetic substrate used in this kit released a fluorescent molecule (AIE) that was measured with a Protopath® fluorometer.17

Another aliquot of PPP was assayed within 4 hours of collection to determine the APTT with a standard technique (General Diagnostics, Organon-Teknika, Durham, North Carolina).

Platelet counts and hematocrit were obtained with a Coulter counter. Fibrinogen concentration was determined after an excess of thrombin was added by measuring the clot formation time (American Dade Test, American Dade, Aguada, Puerto Rico) on a Fibrometer® (B4120-1).

**Statistical Analysis**

Normally two segments per artery per pig were analyzed; the next segment was also included in the analysis when a tear extended beyond the dilated segment. Because of the large differences in platelet and fibrinogen deposition between deep and superficial injury, separate analyses were performed for these two types of injury. Because all segments within a pig were exposed to the same concentrations of heparin, analyses were done using the pig as a unit of the study. Because the variability of platelet and fibrinogen deposition in the same pig or among pigs was directly related to the mean level, a logarithmic transformation was first applied to all platelet and fibrinogen deposition; this removes the dependence of variability on the mean and allows variation to be expressed as a percentage of the mean rather than in absolute terms.

Means of log of number of platelets and molecules of fibrinogen per centimeter squared of deep and superficial injury within each pig were regressed against heparin dosages, heparin concentrations, and units of heparin with linear regression. Presence of thrombus on deeply injured segments was also regressed against heparin dosage and total heparin administered. Differences between means of log platelet and fibrinogen deposition were obtained for animals in the high and low heparin group and the hirudin group. The differences between mean logs of platelet and fibrinogen deposition on deep and superficial injury were analyzed with two-sample Student's t test. To determine the effect of different heparin dosages, differences between groups in heparin concentrations and APTT at each time were analyzed with an analysis of variance. Mean values of hematocrit, plasma fibrinogen, and platelets before the procedure and at the time of death were compared.

**Results**

Table 1 shows the number of pigs and segments with deep and subendothelial injury in each group. The time from the angioplasty procedure to death was 57±12 minutes (range, 46–96) and was not significantly different between groups. Mean units of heparin administered during the procedure and the concentration of heparin at the time of angioplasty are listed in Table 2. These heparin concentrations were achieved 10 minutes after starting the heparin infusion and remained stable throughout the procedure (Figure 1, right). Heart rate and blood pressure were within normal range and did not significantly change throughout the procedure.

**Platelet and Fibrinogen Deposition on Areas of Deep and Subendothelial Injury**

As we have shown previously, platelet deposition in subendothelial injury is always significantly less
TABLE 2. Units of Heparin Administered and Heparin Concentrations* in Each Dosage Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Total heparin (units)</th>
<th>Heparin (units/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,884±281</td>
<td>0.67±0.3</td>
</tr>
<tr>
<td>2</td>
<td>3,898±602</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td>3</td>
<td>7,722±1,071</td>
<td>3.75±0.7</td>
</tr>
<tr>
<td>4</td>
<td>11,790±2,262</td>
<td>6.05±1.1</td>
</tr>
<tr>
<td>5</td>
<td>13,973±1,355</td>
<td>10.3±1.4</td>
</tr>
<tr>
<td>6</td>
<td>17,700±2,506</td>
<td>14.1±1.1</td>
</tr>
</tbody>
</table>

*Immediately before angioplasty (see Figure 1B for heparin concentrations over time).
Values are mean ± SD.

than in deep injury. In this study, mean log of number of platelets per centimeter squared were 1.64±0.39 versus 2.50±0.96 (p<0.0001), and mean log of molecules of fibrinogen per centimeter squared were 2.29±0.56 versus 2.89±0.66 (p<0.0005) for segments with all subendothelial and with at least partial deep injury, respectively. All animals treated with hirudin had deeply injured arteries (Table 1). When platelet and fibrinogen deposition was assessed by the specific area of deep injury within each segment, mean log platelet deposition and log of molecules of fibrinogen per area of deep injury in segments from all pigs treated with heparin were 4.74±1.03 and 5.02±0.64, respectively. Figure 2 (top) shows the number of platelets and molecules of fibrinogen deposited per area of deep injury for each heparin dosage group and hirudin. Log of platelet deposition by area of deep injury showed a significant inverse relation with heparin group (r=−0.56, p<0.0001), total units of heparin administered (r=−0.55, p=0.0003; Figure 3, upper left) and mean plasma heparin concentration in units per milliliter (r=−0.55, p=0.0004; Figure 3, upper right). Similarly, log of molecules of fibrinogen deposited was inversely correlated with heparin dosage (r=−0.47, p=0.002), total units of heparin (r=−0.43, p=0.009; Figure 3, lower left), and mean plasma heparin concentrations (r=−0.44, p=0.009; Figure 3, lower right). Figure 2 (bottom) shows that the number of platelets and molecules of fibrinogen on segments with subendothelial injury was markedly lower than with deep injury and was independent of heparin dosage or plasma concentration. Hirudin significantly reduced platelet and fibrinogen deposition in deeply injured segments; log of platelet deposition per area of deep injury was 3.23±0.44, and log of molecules of fibrinogen deposition was 3.98±0.36. Hirudin reduced platelet and fibrinogen deposition even compared with the highest concentration of heparin (3.8±0.6 and 4.6±0.6, respectively; p<0.04). At similar APTT, hirudin was more effective in reducing platelet and fibrinogen deposition than heparin. Platelet deposition per centimeter squared of deeply injured areas was 5.44±0.60 for animals in the 35 units of heparin group and 3.23±0.44 for the hirudin treated animals (p<0.001); a similar significant difference was observed for a number of molecules of fibrinogen per centimeter squared of deeply injured areas—5.33±0.53 versus 3.98±0.36, respectively (p<0.001) (see Figure 2, top).

**Thrombus**

Increasing dosages of heparin resulted in a reduction of macroscopic thrombus on deeply injured segments. All seven pigs in group 1 that had segments with deep injury developed macroscopic thrombus, while only one animal of six in the highest heparin group had a thrombus in two deeply injured segments. No thrombus was seen in any of the pigs treated with recombinant hirudin. Figure 4 shows the percentage of segments with deep arterial injury that had thrombi. A linear regression of the proportion of deeply injured segments with thrombus versus heparin dosage as a continuous variable showed a significant reduction with increasing dosage (r=−0.43, p=0.003) and with increasing total units of heparin administered (r=−0.41, p=0.008).

**Laboratory Data**

Heparin concentrations for each dosage group were stable from 10 minutes after starting the infusion to death (Figure 1 right; Table 2). The differ-

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**Figure 1.** Left panel: Heparin concentrations after a single bolus of heparin. The concentrations at 10 minutes after the bolus steadily declined; by 30–40 minutes, they were reduced by 50%. Right panel: Mean heparin concentrations for each group. These concentrations were achieved by a bolus followed by a continuous infusion until death and were stable throughout the procedure.
ences in heparin concentrations between groups were significant at each determination \( (p=0.0001) \). APTT assayed 10 minutes after starting the heparin infusion were at least threefold that of control basal values for animals in the lowest group (35 units/kg); that ratio increased almost geometrically so that it was higher than 3 minutes for groups 2–6; the ratio in each group remained similar from 10 minutes posttreatment to death. APTT in animals treated with hirudin increased from 2.5 to 3.5 of their basal control values. The differences in APTT between groups were significant and persisted until death \( (p=0.007) \). There was a good direct correlation between average concentration of heparin with average APTT for all the groups together \( (r=0.49, p=0.0005) \). AT III did not change significantly at death when compared with basal value in the animals treated with heparin.

Hematocrit, platelet count, and fibrinogen levels were assessed before administering heparin and before death. There was a slight nonsignificant reduction in hematocrit for all animals from 25±3.3% to 22.6±3.2%, platelet counts from 463.1±111.7×10^3/μl to 400.9±107.1×10^3/μl, and fibrinogen levels from 164±36.2 to 146.7±34 mg/dl (11% reduction). Hematocrit, platelet counts, and fibrinogen at death

**FIGURE 2.** Top panel: Mean platelet and fibrinogen deposition per centimeter squared of deep arterial injury. Vertical lines are the SD of the mean. Values are expressed as the natural log on the left vertical axes and the natural numbers on the right vertical axes. Platelet and fibrinogen deposition in animals treated with hirudin were significantly lower compared with the ones in the highest heparin (250) group. (See Figure 1 for plasma heparin concentrations.) Bottom panel: Mean platelet and fibrinogen depotsions per centimeter squared of subendothelial injury.

**FIGURE 3.** Left panel: Relation between total units of heparin administered and log of platelet deposition (upper) and log of molecules of fibrinogen (lower). The significant inverse relations are shown. Right panel: Relation between heparin concentration in units per milliliter and log of platelet deposition (upper) and log of molecules of fibrinogen (lower). The significant inverse correlations are shown.
were not significantly different between treatment groups. In animals treated with hirudin, there was also a minor nonsignificant reduction of hematocrit from 30.8 ± 3.6% to 27 ± 3% and platelet counts from 453.4 ± 90 × 10⁹/µl to 441.8 ± 97.5 × 10⁹/µl. Fibrinogen levels decreased from 180 ± 27 to 148 ± 21 mg/dl (17% reduction; p = 0.04). An average of 950 ml 5% dextrose was administered to pigs treated with hirudin and 1,100 ml to pigs treated with hirudin during flushing of hemodynamic lines.

**Discussion**

Our results support the hypothesis that platelet and fibrinogen deposition and mural thrombosis decrease with increasing antithrombin activity (with increasing heparin dosages and blood levels and with hirudin) achieved immediately before the deep arterial injury. The percentage of deeply injured arterial segments with macroscopic mural thrombus decreased from 75% to 10% as the heparin dosage increased. Platelet and fibrinogen depositions were lowest and mural thrombosis totally prevented in all the animals treated with hirudin. The results of this prospective randomized study extend the results of our retrospective study⁵ and show that increasing antithrombin activity plays a major role in reducing platelet deposition and mural thrombosis after deep arterial injury. The major effect of heparin, its antithrombin effect, is incompletely expressed at the usual therapeutic doses but better seen at higher doses. This is supported by the similar but more marked effect of hirudin, a specific receptor inhibitor of the coagulation enzyme, thrombin.

Hirudin prevents fibrinogen clotting and thrombin catalyzed activation of factors V, VIII, and XIII and thrombin-induced platelet activation.¹⁸,¹⁹ Thrombin generation is markedly amplified (280,000-fold)³ by formation of the prothrombinase complex that is formed by the binding of factors Va, Xa, and Ca²⁺ to the phospholipid of the platelet membrane. Thus, hirudin by inhibiting activation of factor V can markedly suppress thrombin generation and the powerful coagulation and positive feedback effects of thrombin on the activation of coagulation factors and platelets. Thrombin induces platelet aggregation through a direct mechanism that is independent of ADP and thromboxane A₂. In addition, thrombin promotes the stabilization of these platelet aggregates by cleaving fibrinogen to fibrin and by the activation of factor XIII, which cross-links fibrin.²⁰ Because of the potent and amplifying effects of thrombin relatively early in the coagulation cascade and on platelets, the primary goal of specific and more complete inhibition of thrombin to prevent platelet-thrombus formation after acute angioplasty is logical.

In previous studies, we investigated the usefulness of blocking other pathways of platelet activation during acute arterial angioplasty in our pig model. While using a 100 units/kg single bolus of heparin just before angioplasty, pretreatment with low dose aspirin (1 mg/kg/day) significantly reduced ¹¹In-labeled platelet deposition and the incidence of macroscopic thrombus after deep arterial injury to only 36%;¹¹ however, a thromboxane A₂ receptor blocker (SQ 29548), ketanserin (a type II serotonin antagonist), or both combined did not significantly reduce ¹¹In-labeled platelet deposition or mural thrombosis compared with placebo.²¹ Thus, our results suggest that maximal inhibition of thrombin during deep arterial injury is more effective than lower dose heparin or heparin plus blockers of cyclooxygenase, thromboxane receptors, serotonin receptors, or both of the latter pathways of platelet activation in our model. So far, thrombin inhibition with hirudin has been the best therapeutic agent in our model.

To achieve stable plasma levels of heparin after the initial bolus, we immediately started a continuous infusion. This maintained steady plasma levels throughout the study. A rapid decrease to less than half the initial plasma level 30 minutes after a single bolus has been described in humans,²² and we have shown this in the pig (Figure 1, left). This has important clinical implications because most patients who undergo coronary angioplasty usually receive only a bolus of heparin when catheters are inserted; however, the time elapsed until the angioplasty is performed and finally completed is variable. Thus, at the moment of the initial and especially the last balloon inflation and arterial injury, heparin levels after a single bolus are likely to be suboptimal, thereby increasing the risk of acute thrombosis and probably restenosis. In the present study, the dosage of heparin was deliberately increased far beyond the usual therapeutic range in humans to determine whether increased anticoagulant effects might further reduce the incidence of arterial thrombus formation.

All partial thromboplastin times were increased at least threefold the control basal values in the 35 units/kg group; in the other five groups, they were almost always greater than 180 seconds. Heparin
levels achieved in the lowest heparin dosage (35 units/kg) are considered within therapeutic range in humans (see Table 2). Hirudin prolonged the APTT to only threefold that of the basal value or to the same degree as 35 units/kg heparin; however, the antithrombotic effects of hirudin (especially against mural thrombosis and also quantitative platelet and fibrinogen deposition) are markedly greater than heparin (see Figures 4 and 2, top), probably due to its more specific and potent antithrombin activity.

Although we have used large heparin dosages that causes a prolongation of the APTT to greater than 180 seconds, there was no evidence of acute bleeding, even at the arterial cut-down sites. There was a minimal decrease of hematocrit, platelet count, and fibrinogen levels with increased heparin dosages, as well as with hirudin. However, these reductions in these experiments were not statistically significant, of no clinical importance, and probably related, at least in part, to hemodilution. Because the decrease in platelet count was less than 10%, a direct effect of heparin on lowering the platelet count is very unlikely. In this acute experiment, the plasma levels of antithrombin III were stable throughout the procedure and not affected by different heparin dosages; a reduction might be expected with longer durations of heparin therapy.23,24

In conclusion, in this porcine model, the number of platelets, molecules of fibrinogen, and incidence of mural thrombus were inversely related to heparin levels at the time of acute angioplasty. Higher dosages of heparin appear necessary to reduce arterial thrombosis than are customarily recommended in venous thromboembolic disease. The marked beneficial effects of the specific thrombin receptor inhibitor hirudin (better than the highest dosage of heparin administered) suggest the need for potent specific thrombin inhibition during deep arterial injury such as during angioplasty or endarterectomy or syndromes involving acute arterial injury such as unstable angina or acute myocardial infarction or after thrombolysis for acute myocardial infarction.25,26 More specific antithrombin drugs with less secondary bleeding effects than heparin need further evaluation because of the striking beneficial effects obtained with specific thrombin inhibition.

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References


**KEY WORDS**
*anticoagulation* • arterial injury • heparin • hirudin • thrombin • thrombosis • angioplasty
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