Hirudin, Heparin, and Placebo During Deep Arterial Injury in the Pig
The In Vivo Role of Thrombin in Platelet-Mediated Thrombosis

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Three dosages (0.3, 0.7, and 1.0 mg/kg) of recombinant hirudin, a specific inhibitor of thrombin, were compared with heparin (50 units/kg) and placebo for reducing thrombus formation in the carotid arteries of 50 pigs after deep injury by balloon dilatation. Each drug was administered as a bolus followed immediately by a continuous infusion of the same dose per hour. Major end points were quantitative indium-111–labeled platelet and iodine-125–labeled fibrinogen deposition and the incidence of mural thrombosis. This study showed that heparin, at a dose that prolonged the activated partial thromboplastin time (APTT) to twice the control time, did not prevent mural thrombosis or significantly reduce platelet deposition compared with placebo but did reduce fibrinogen deposition. Recombinant hirudin markedly reduced platelet and fibrinogen deposition in a dose-related manner and totally eliminated mural thrombosis at an APTT of two to three times that of control. Platelet deposition (×10^4/cm^2, mean±SEM) in areas of deep arterial injury for the placebo, heparin, and 0.3, 0.7, and 1.0 mg/kg hirudin groups was 54±21, 33±9, 22±6, 8±1, and 7±1, respectively; electron microscopy showed a single layer (or less) of platelets at the two highest hirudin dosages. The incidence of macroscopic mural thrombosis was 76% with placebo, 57% with heparin, 46% with 0.3 mg/kg hirudin; there were no thrombi with 0.7 or 1.0 mg/kg hirudin (p<0.01). The APTT is a valuable index of the plasma hirudin concentration (r=0.89, p<0.001) and correlated inversely with quantitative platelet (r=-0.67, p<0.0001) and fibrinogen deposition (r=-0.42, p=0.005). The thrombin time is overly sensitive and plays no role in monitoring heparin or hirudin therapy for arterial thrombosis. Heparin and all dosages of hirudin abolished platelet aggregation to thrombin and prolonged the bleeding time to a similar degree; neither test reflects the in vivo antithrombotic efficacy of heparin or hirudin. Thrombin plays a critical role in arterial thrombosis. Heparin in conventional doses does not significantly reduce arterial platelet thrombosis. Hirudin produces a dose-dependent reduction in platelet and fibrinogen-fibrin deposition, which correlates with the prolongation of the APTT and the hirudin plasma level. Hirudin totally eliminates macroscopic mural thrombus formation at dosages that prolong the APTT to at least twice control and is a promising therapeutic agent for platelet-rich arterial thrombi. (Circulation 1990;82:1476–1484)

Balloon dilatation produces mechanical disruption of the arterial wall with injury into the media that releases tissue thromboplastin (tissue factor) and exposes very thrombogenic deep arterial structures (mainly smooth muscle cells and collagen types I and III) to flowing blood.1–3 This procedure, even in normal arteries, triggers activation of the coagulation system and platelets that together with the arterial wall matrix generate large amounts of thrombin by the thrombinase

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complex. Thrombin binds to the arterial wall matrix and platelet membrane receptors and produces platelet activation and subsequent arterial thrombosis. We previously showed a dose-dependent reduction of mural thrombosis and platelet and fibrinogen deposition by six incremental doses of heparin after deep arterial injury. Recombinant hirudin, a specific thrombin inhibitor, reduced platelet deposition to levels lower than did the highest dose of heparin, completely prevented mural thrombosis, and appeared more effective than heparin plus low-dose aspirin or receptor blockade of thromboxane and serotonin. This suggested the critical role of thrombin inhibition in the action of hirudin during arterial thrombosis.

Anticoagulation to levels used in venous thrombosis is assumed to be effective in arterial thrombosis. However, arterial thrombi are platelet rich at the site of deep injury and may not be affected by conventional anticoagulant dosages. Heparin has multiple actions and can accelerate inhibition of thrombin and factors IXa, Xa, and XIa by antithrombin III. Hirudin acts differently, directly and highly specifically to bind thrombin without inhibition of other proteases. Because hirudin reacts stoichiometrically in a 1:1 ratio with thrombin with a very low dissociation constant, the amount of drug needed to prevent thrombosis should be an indirect measure of the amount of thrombin generated.

The aims of the current study, in a deep arterial injury model, were to test whether 1) a conventional dosage of heparin will reduce platelet deposition and the incidence of mural thrombosis compared with placebo, 2) hirudin will produce a dose-dependent reduction in quantitative platelet and fibrinogen deposition and the incidence of mural thrombosis; the lowest effective activated partial thromboplastin time (APTT) prolongation for the prevention of arterial thrombosis can be established, 3) the APTT during hirudin therapy will correlate inversely with the platelet and fibrinogen deposition, and the incidence of mural thrombosis; the APTT and the thrombin time will provide a direct measure of hirudin blood levels; and 4) doses of heparin and hirudin sufficient to inhibit platelet aggregation to thrombin and prolong the bleeding time will reduce platelet deposition and mural thrombosis compared with placebo.

We evaluated recombinant hirudin at three different dosages: 0.3 mg/kg, used in venous thrombosis but not tested during arterial injury; 1.0 mg/kg, effective in five pigs in our preliminary study; and 0.7 mg/kg, an intermediate dosage. Hirudin was compared with heparin, at a dosage known to prolong the APTT to twice control, and with placebo.

**Methods**

The 50 normal pigs of Babcock four-way cross stock (a mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) consisted of 40 males and 10 females and were approximately 4 months old with a mean weight of 37.7±0.5 kg (range, 32–48 kg). They were randomly allocated to one of five treatment groups: placebo (0.9% saline), sodium heparin at 50 units/kg (from porcine intestine that contained 1,000 USP units/ml) or recombinant desulfato hirudin (CGP 39393; sequence of hirudin variant 1 but lacks sulfate on tyrosine 63; specific activity, 11,496 ATU/mg; known as hirudin in the text) at 0.3, 0.7, or 1.0 mg/kg. The active drugs or matching saline were given as a bolus (units/kg or mg/kg) followed immediately by a continuous infusion of the same dose per hour. Although drug administration during the balloon dilatation procedure was not blinded, all subsequent tissue and sample analysis was performed blinded to the treatment administered. This study was approved by the Mayo Clinic Animal Care Committee and conformed to American Heart Association guidelines.

**Experimental Protocol**

This model of carotid arterial injury has been described in detail previously. Autologous platelets were labeled with 300 μCi In troponone. The platelets were re-injected together with 250 μCi I-labeled human fibrinogen on the day before the procedure. On the day of surgery, the pigs were sedated with ketamine (Ketaset, Bristol Laboratories, Syracuse, N.Y.), intubated and mechanically ventilated with room air (Harvard respirator, Harvard Apparatus, South Natick, Mass.) mixed with 0.5% halothane (Fluothane, Wyeth-Ayerst Laboratories, Philadelphia, Pa.) to maintain anesthesia. The electrocardiogram and intra-arterial pressure were continuously monitored throughout the procedure.

Both femoral arteries were dissected; via the left femoral artery, an 8 mm×3-cm polyethylene angioplasty catheter (Medi-tech, Watertown, Mass.) was advanced under fluoroscopic guidance to the left common carotid artery for arterial dilatation. A 14G Angiocath was placed in the right femoral artery to obtain blood for platelet count, fibrinogen, hematocrit, APTT, thrombin time (TT), antithrombin III (AT III, only in animals given heparin), and drug plasma concentrations. Another Angiocath was placed in the left femoral vein for drug administration and sampling for “ex vivo” whole blood platelet aggregation. All the lines for blood sampling were constantly flushed with 5% dextrose. After the lines were in place, a basal bleeding time was performed in the ear using a standardized method. The treatment bolus of saline, heparin, or hirudin was then given and followed immediately by the infusion (in 0.9% NaCl), administered with a Harvard pump at a rate of 0.8 ml/min. Ten minutes after starting the infusion, another bleeding time was performed in the other ear.

Carotid angioplasty of the left and then the right common carotid arteries was then performed between the first and third cervical vertebrae using a standardized procedure (five inflations for 30 seconds each at 6 atm, with 60 seconds between inflations). Fifteen minutes after the last balloon...
inflation in the right common carotid artery, 120 ml 0.5% Evans Blue dye in 0.9% NaCl were injected into the descending aorta. The animals received an overdose of pentobarbital and were sacrificed. The proximal descending aorta was immediately cannulated and the carotid arteries flushed with normal saline and then perfused at physiologic pressure with 2% glutaraldehyde for 15 minutes. Both carotid arteries were harvested; after being cleaned of all adventitia, the dilated portion was divided into two equal segments and two similar-sized segments were taken proximal and distal to the dilated area.

**Tissue Analysis**

The number of platelets and the molecules of fibrinogen (some incorporated into fibrin) deposited on the arterial segments were quantitated by the method of Dewanjee et al.\textsuperscript{15,16} Counting for \textsuperscript{111}In was performed on the day of surgery and \textsuperscript{125}I 2–3 weeks later, after the \textsuperscript{111}In had decayed.

After counting the radioactivity, all segments were cut open and pinned onto a paraffin block to be photographed. From color photographs, computer-assisted planimetric measurements of the area of deep injury (if it was present) and the total segment area were obtained for all segments.\textsuperscript{7,17} Two rings from each arterial segment were stained with hematoxylin-cosin and van Gieson stains. Deep arterial injury (a tear through the internal elastic lamina into the media) or subendothelial injury (endothelial denudation with preservation of the internal elastic lamina) was documented by two independent observers. A twofold magnifying lens was used to examine for the presence of macroscopic mural thrombus.

Segments with deep and subendothelial arterial injury were taken from each treatment group, coated with carbon and gold-palladium alloy, and examined with a scanning electron microscope (Autoscan, ETEC Corporation, Hayward, Calif.). Representative areas were photographed and evaluated by two investigators.

**Laboratory Tests**

All blood samples were collected with the two-syringe technique. Before treatment, blood was drawn for basal platelet count, fibrinogen, hematocrit, AT III, APTT, TT, and drug concentrations. APTT, TT, and drug levels were measured 10 minutes after starting the drug infusion, before each carotid angioplasty and immediately before sacrifice. At sacrifice, samples were again obtained for platelet count, fibrinogen, hematocrit, and AT III.

Platelet counts, hematocrit, APTT, TT, and fibrinogen were determined immediately as previously reported.\textsuperscript{7} Blood for drug levels and AT III was mixed 9 parts to 1 with a 3.8% trisodium citrate solution, centrifuged to obtain platelet-poor plasma (PPP), and stored at −20°C until the assays were performed at a batch. Heparin plasma levels were determined with the Stachrom X kit (American Bioproducts Company, Parsippany, N.J.). PPP was incubated with AT III, factor Xa, and buffer, the chromogenic substrate CBS 31.39 was added, and the optical density of the mixture was read in a Kinetic Microplate Reader, V Max (Molecular Devices, Palo Alto, Calif.) connected to an IBM computer. Similarly, hirudin plasma levels were determined by mixing PPP with thrombin and buffer, adding the chromogenic substrate CBS 34.47 (American Bioproducts Co.) and reading the optical density. Another aliquot of frozen PPP was used to measure AT III levels using the Stachrom AT III kit (American Bioproducts Co.). Two aliquots of fresh PPP were assayed within 4 hours of collection for APTT and TT using a standard technique (General Diagnostics, Organon-Teknika Corp., Durham, N.C.).

Aggregation studies were performed on whole blood using a Chrono-Log 560VS Whole Blood Lumi-Aggregometer and a Chrono-Log 705 recorder (Chrono-Log Corp., Havertown, Pa.). Samples were drawn before therapy, immediately after dilatation of the left carotid artery, and at sacrifice. After discarding the first 3 ml, blood was drawn directly into a syringe containing 3.8% trisodium citrate in 1 volume of citrate to 9 volumes of blood. One-milliliter aliquots of citrated blood were placed in plastic cuvettes and incubated at 37°C for 10 minutes. A Teflon-coated stir bar and an electrical impedance electrode were inserted into the cuvette. After a 60-second baseline recording and calibration (80 chart paper units, 20 Ω), 1–10 μl of agonist was added to the cuvette, and the aggregation curve was recorded until the maximum aggregation was achieved or for at least 5 minutes. The concentration of each agonist was titrated against the pretreatment sample to determine the minimum dose that would give approximately 20% deflection from baseline. The following agonists were used: ADP (Chrono-Log) 2.5–15.0 μM final concentration, collagen (Chrono-Log) 1.0–2.5 μg/ml final concentration, and thrombin (Parke-Davis Thrombostat) 1.0–3.0 units/ml final concentration. Posttreatment samples were run at the concentration of agonist previously determined from the pretreatment sample. The rate of aggregation was assessed from the slope at the steepest portion of each curve.

**Statistical Analysis**

Results are expressed as mean±SEM. Two segments per artery per animal were analyzed. In one animal, an additional segment was included because the tear extended past the dilated region. Because of the high variability of platelet and fibrinogen deposition and because of use of the animal as the unit of study (because all segments in a pig were exposed to the same therapy), analysis was performed on the natural log of these values averaged over all deeply injured segments in each animal. The area of deep injury cannot be controlled and has been found to correlate with platelet and fibrinogen deposition.\textsuperscript{7,17} Analysis of covariance was used to adjust for the average area of deep injury before testing for differences between dosage groups in platelet and fibrin-
Table 1. Platelets and Molecules of Fibrinogen/Fibrin Deposited and Proportion of Segments With Mural Thrombosis in Areas of Deep and Subendothelial Injury by Treatment Group

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Heparin 50 (units/kg)</th>
<th>Hirudin (mg/kg)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td>0.7</td>
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<tr>
<td>Platelet deposition</td>
<td></td>
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<tr>
<td>(×10^12/cm^2)</td>
<td></td>
<td></td>
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<tr>
<td>Deep injury</td>
<td>54±21</td>
<td>33±9</td>
<td>22±6</td>
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<tr>
<td>Subendothelial injury</td>
<td>4.4±1.2</td>
<td>3.3±0.7</td>
<td>3.0±0.4</td>
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<tr>
<td>Fibrinogen deposition</td>
<td></td>
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<tr>
<td>(×10^12/cm^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep injury</td>
<td>54±24</td>
<td>19±2*</td>
<td>28±6</td>
</tr>
<tr>
<td>Subendothelial injury</td>
<td>4.0±0.2</td>
<td>4.8±0.9</td>
<td>5.6±0.5</td>
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<tr>
<td>Mural thrombus**</td>
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<tr>
<td>Deep injury</td>
<td>19/25 (76%)</td>
<td>13/23 (57%)</td>
<td>13/29 (45%)</td>
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*p<0.05 compared with placebo; †p<0.05 compared with heparin (50 units/kg); ‡p<0.05 compared with hirudin (0.3 mg/kg).

**Number of segments with thrombus/total number of segments with deep injury p<0.01 between groups; no segment with subendothelial injury had mural thrombus.

Ogen deposition. For segments with superficial injury, group differences in the log of platelet and fibrinogen deposition were adjusted for the total segment area and compared using analysis of variance.

Analysis of variance was also used to determine whether there was a difference between the dosage groups in the average of the preright and the preleft APTT. Individual group means were compared by the Newman-Keuls test.

Pearson’s x^2 test was used to test for an overall difference between groups in the incidence of thrombus. The mean log of platelet and fibrinogen deposition per square centimeter of total area in deeply injured segments was correlated with the hirudin dosage, the total milligrams administered and the APTT using the Spearman rank correlation coefficient.

**Results**

Mean procedural time from induction of anesthesia to sacrifice was 1.2±0.2 hours and did not differ between groups. Heart rate and blood pressure remained stable throughout the procedure apart from one animal in the 0.7 mg/kg hirudin group that developed a supraventricular tachycardia and then died of electromechanical dissociation before carotid dilatation. No drug effect was observed in the hemodynamic variables. There were no bleeding complications despite bilateral femoral cutdowns and cannulation of blood vessels.

**Platelet and Fibrinogen Deposition**

A deep injury occurred in 76% of segments in the dilated region; the rest had subendothelial injury. When the number of platelets was adjusted for the area of deep injury, platelet deposition in animals treated with 0.7 and 1.0 mg/kg hirudin was significantly lower than those treated with placebo, 50 units/kg heparin, and 0.3 mg/kg hirudin that were not significantly different from each other (Table 1). There was no difference between 0.7 and 1.0 mg/kg hirudin. For animals treated with hirudin, there was an inverse correlation between platelet deposition and the dosage group (r=−0.78, p<0.0001), the total amount of hirudin administered (r=−0.63, p<0.0001) and the APTT (r=−0.67, p<0.0001) (Figure 1).

Table 1 also lists the number of molecules of fibrinogen deposited by treatment group and type of injury. Fibrinogen deposition was significantly lower in the 0.7 and 1.0 mg/kg hirudin groups compared with animals treated with placebo and 0.3 mg hirudin (p<0.01); fibrinogen deposition was also significantly lower (p=0.02) in the 1.0 mg/kg hirudin group compared with heparin, and slightly lower with 0.7 mg hirudin than hirudin (p=0.16). Heparin reduced fibrinogen deposition compared with placebo (p=0.04). For animals treated with hirudin, there was an inverse correlation between fibrinogen depo-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Platelet deposition in segments with deep arterial injury versus total amount of hirudin administered (in hirudin-treated animals) and activated partial thromboplastin time (APTT) (all animals).
sition and the dosage group \( r = -0.63, p < 0.0001 \), the total amount of hirudin administered \( r = -0.38, p < 0.05 \) and the APTT \( r = -0.42, p = 0.005 \) in areas of deep arterial injury. Platelet and fibrinogen deposition on areas of subendothelial injury was very low and did not significantly change with treatment.

Figure 2 shows scanning electron photomicrographs from areas of deep and subendothelial injury in representative animals, one treated with 1.0 mg/kg hirudin and the other with placebo. In the area of deep arterial injury there is less than a platelet monolayer in the animal treated with hirudin (Figure 2D), compared with many layers of platelets in the control animal (Figure 2C). Moreover, the number of platelets and their shape after hirudin treatment (single platelets with spreading but no aggregation) are similar to those found after subendothelial injury.

**Mural Thrombus**

In segments with deep arterial injury, there was macroscopic mural thrombosis in 76%, 57%, and 45% of the segments in the placebo-, 50 units/kg heparin-, and 0.3 mg/kg hirudin-treated groups, respectively. No thrombus was present in animals that received 0.7 or 1.0 mg/kg hirudin or when the APTT was prolonged more than twice control (Figure 3). The difference between groups in the incidence of mural thrombi was significant \( p < 0.01 \).

**Laboratory Data**

Drug concentrations were stable during the procedure with a slight peak 10 minutes after the initial bolus (Figure 4A). The same stability was also present in the APTT prolongation (Figure 4B). At the time of angioplasty, mean hirudin concentrations were 3.6±0.6, 7.1±0.5, and 7.9±0.1 \( \mu \)g/ml for 0.3, 0.7, and 1.0 mg/kg, respectively. The hirudin level correlated well with the prolongation of the APTT (Figure 5). The heparin level was 0.8±0.1 units/ml. The APTT was prolonged to twice the control value in animals treated with 50 units/kg heparin and 0.7

**Figure 2.** Scanning electron photomicrograph of the injured luminal surface of the common carotid artery (original magnification, \( \times 1,000 \)) from animals treated with placebo (panel A) and 1.0 mg/kg hirudin (panel B). The right side of each panel has subendothelial injury (SI) and the left side has deep injury (DI). Panel C: A higher magnification (\( \times 3,000 \)) of the platelet thrombus covering the area of deep injury in panel A (placebo). Panel D: Higher magnification (\( \times 3,000 \)) of the area of deep injury from panel B (hirudin treated), with very few adherent platelets, similar to areas of subendothelial injury.

**Figure 3.** Activated partial thromboplastin time (APTT) prolongation \( (\times \) basal value), measured in each hirudin-treated pig (as the average of samples taken immediately before the left and right carotid dilatations), versus absence or presence of macroscopic mural thrombus. Note the total absence of mural thrombus when the APTT prolongation is 2.0 \( \times \) basal value or greater.
mg/kg hirudin, 1.7 times control in the animals given 0.3 mg/kg hirudin and three times control in those treated with 1.0 mg/kg hirudin (Figure 4B).

Basal AT III in animals that received heparin were 93.9±1.8% and did not change at sacrifice. Thrombin time (20 seconds at baseline) was greater than 180 seconds for all actively treated animals, regardless of the drug dosage. All pigs had a normal bleeding time (3 minutes or less) before drug administration; 10 minutes after starting the infusion, all bleeding times were equally prolonged with active treatment (Figure 6). It was noted that in some animals treated with heparin or hirudin that hemostasis occurred after several minutes but was followed by rebleeding 30–90 seconds later and appeared to reflect decreased fibrin stabilization of the hemostatic plug.19 No drug effect was observed on platelet counts or fibrinogen.

“Ex Vivo” Aggregations

The rate of platelet aggregation to ADP and collagen did not change after treatment with heparin or hirudin and was similar to placebo. The basal mean rate of platelet aggregation with thrombin was 2.7±1.5 minutes and did not differ between treatment groups; after treatment, aggregation to thrombin was completely abolished with heparin or hirudin but was unchanged with placebo.

Discussion

This in vivo study of deep arterial injury demonstrates that 1) a conventional dose of heparin (APTT prolonged to twice control) does not prevent mural thrombus or significantly reduce platelet deposition compared with placebo, but does reduce quantitative fibrinogen/fibrin deposition, 2) recombinant hirudin, which has an entirely different mechanism of action than heparin, markedly reduces platelet and fibrinogen/fibrin deposition and mural thrombus formation in a dose-related manner without causing bleeding complications, 3) the APTT is a valuable index of the plasma hirudin level and correlates inversely with quantitative platelet and fibrinogen/fibrin deposition and the incidence of mural thrombosis; the thrombin time is overly sensitive and plays no role in monitoring therapy with heparin or hirudin in arterial thrombosis, and 4) heparin and all dosages of hirudin abolished platelet aggregation to thrombin and prolonged the bleeding time to a similar degree; neither test reflects the in vivo antithrombotic efficacy of these drugs.

The effective dose of hirudin that totally eliminates mural thrombosis prolongs the APTT two to three times control. The maximum effects of hirudin were
observed at 0.7 and 1.0 mg/kg; pigs given these dosages had only a single layer of platelets deposited and no visible thrombus. Electron microscopy of vessels from these hirudin-treated animals showed that the extent of platelet deposition on areas of deep arterial injury appeared similar to the platelet deposition on areas of subendothelial injury; there was a single layer (or less) of activated spreading platelets without aggregation. A dosage of 0.3 mg/kg hirudin was significantly less effective in this arterial model.

Aspirin, which inhibits platelet aggregation to prostaglandins only, is a weak antagonist, and reduces platelet deposition to a lesser degree; 25% of vessels in aspirin-treated animals still have mural thrombosis.\(^5,8\) Thromboxane A\(_2\) receptor blockade, serotonin receptor blockade or both do not significantly reduce platelet deposition or mural thrombosis in the presence of deep arterial injury.\(^9\) Platelet aggregation studies also document that hirudin specifically inhibits platelet activation by thrombin and confirm previous extensive pharmacological studies documenting the specific antithrombin effects of hirudin.\(^10-14\) Thus, platelet thrombosis in arteries appears to be largely thrombin mediated.

The powerful and specific antithrombin action of hirudin compared with heparin is shown by the differences in platelet and thrombus deposition in pigs treated with 50 units/kg heparin and 0.7 mg/kg hirudin, which both prolonged the APTT to twice the control value. Thrombosis in the heparin-treated animals was present in 57% of segments, whereas no thrombus was found in the hirudin-treated group. Quantitative platelet deposition was markedly decreased and limited to a single layer (or less) by hirudin compared with extensive, multilayered platelet deposition in pigs treated with heparin.

Although both heparin and hirudin are antithrombin drugs, their interaction with thrombin and efficacy in arterial thrombosis are very different. Heparin is a cofactor of plasma antithrombin III; heparin-antithrombin III blocks thrombin and prevents fibrin formation but, unlike hirudin, is inhibited by fibrin II monomer and platelet factor 4.\(^4\)\(^20\) Heparin is 50 times less effective at partially inhibiting fibrin-bound compared with free thrombin. However, hirudin totally inhibits fibrin-bound and free thrombin at the same dosage.\(^21\) In addition, formation of the prothrombinase complex, with binding of Xa to the activated platelet in association with Va, protects Xa from inactivation by AT III.\(^22\) Heparin was almost as effective as 0.7 mg/kg hirudin at reducing fibrinogen deposition. The ability to reduce the formation of fibrin may explain the beneficial anticoagulant effect of heparin in the low shear venous system where fibrin formation is prominent. This study confirms that heparin dosages that may be adequate in the venous system are insufficient for high-shear, platelet-rich arterial thrombi.

In contrast, hirudin interacts directly with thrombin and induces significant conformational changes in the thrombin molecule, causing the loss of its coagulant activity.\(^23\) Thrombin activates platelets in part by binding glycoprotein Ib on the platelet membrane and by producing a proteolytic degradation of glycoprotein V.\(^24\) The site where hirudin binds thrombin appears important for the reaction of thrombin with platelets that in turn affects platelet activation.\(^25\)

Hirudin has a high affinity for thrombin \(K_a = 10 \times 10^{-12}\) and the platelet-thrombin receptor (approximately 10–100 times higher than the \(K_a\) for thrombin). Hirudin can displace thrombin from its binding sites on platelets,\(^25\) and thus might cause deaggregation. Hirudin is about one tenth the size of the heparin-AT-III complex and appears to have greater access than the heparin-AT-III complex to the thrombin receptor. Hirudin also lacks a natural and local inhibitor in platelets (platelet factor 4 from \(\alpha\)-granules inhibits heparin) or at the surface of thromb (such as fibrin II monomer). Because no naturally occurring inhibitor has been described, the dosage of hirudin administered is the only limiting factor in blocking thrombin. Conversely, the effectiveness of hirudin is strong evidence that thrombin-induced platelet activation is important in the arterial system. The kinetics of destruction of the complexes with hirudin versus heparin-AT-III and other possible “benefits” of the thrombin-hirudin complex, such as prevention of nonenzymatic interactions with thrombin, are unknown.

This study also shows that the APTT can be used as an index of the plasma hirudin concentration. The APTT evaluates the intrinsic coagulation system and reflects deficiencies in or decreased enzymatic action on fibrinogen; prothrombin; factors X, V, VIII, IX, XI, and XII; high molecular weight kininogen, and prekallikrein. Because hirudin inhibits thrombin but does not directly affect other steps in the intrinsic pathway, the APTT can be used as a marker of thrombin inhibition; it shows a linear correlation with plasma hirudin levels at the dosages tested. This, in turn, provides a measure of the antithrombin effect of hirudin on platelets that is dose dependent and clinically significant after the APTT is prolonged to twice the control level. The thrombin time is overly sensitive as previously documented\(^12\) and plays no role in monitoring therapy with heparin or hirudin.

The bleeding time evaluates interaction between the platelet and the arterial wall and effective formation of the platelet plug; it reflects platelet function and number and platelet stabilization by fibrin. A sufficient antithrombin effect prolongs the bleeding time by affecting both platelet function and stabilization by fibrin. A dose response was not seen for hirudin probably because extremes of dosage (no-effect dosage and excessively high dosage) were not used. At the dosages tested, hirudin and heparin prolonged the bleeding time to a similar extent, although no effect on bleeding (either femoral or carotid) was found in treated compared with control pigs. In humans, lower dosages of hirudin (0.1 mg/kg) do not significantly prolong the bleeding time, suggesting that the prolongation may be dose
dependent. At therapeutic dosages, heparin may prolong the bleeding time in humans (M. Verstreken, personal communication).

Other studies using different synthetic antithrombin peptides have also documented prevention of thrombosis in a high shear rate system. In a model of arterial vascular graft thrombosis, Hanson and Harker showed that treatment with d-phenylalanyl-L-prolyl-L-arginyl chloromethyl ketone (FPRCH2CL or ‘P-PACK’) completely prevented vascular occlusion in markedly reduced platelet deposition. Another group, using a different synthetic thrombin inhibitor, reported a dose-dependent inhibition of arterial thrombosis in rats and prevention of occlusion in extracorporeal arteriovenous shunts, Argatroban, yet another thrombin inhibitor, was recently found to be more effective than heparin at reducing platelet-thrombus in a rabbit everted femoral artery model.

In conclusion, deep arterial injury by balloon dilation activates the coagulation system and platelets with thrombin generation, which can be inhibited by hirudin in a dose-dependent manner. The efficacy of hirudin at reducing platelet-thrombus deposition correlates with the hirudin plasma level and the prolongation of the APTT. Heparin therapy, at a dose that prolongs the APTT to twice normal, does not prevent mural thrombosis or significantly reduce platelet deposition. Hirudin is more effective than heparin at preventing acute arterial platelet-rich thrombosis at dosages that produce a similar prolongation in the APTT (two to three times control); platelet deposition is limited to a single layer with no hemodynamic abnormality or overt bleeding. Hirudin has considerable promise for the management of the acute coronary syndromes and other thrombotic disorders.

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