Comparison of Hirudin and Heparin as Adjuncts to Streptokinase Thrombolysis in a Canine Model of Coronary Thrombosis

Dean F. Rigel, Richard W. Olson, and Rodney W. Lappe

Recombinant desulfatohirudin (HI), a potent and specific thrombin inhibitor, was compared with heparin (HE) as an adjunct to streptokinase thrombolysis. In pentobarbital-anesthetized dogs, an occlusive thrombus (whole blood + thrombin) was introduced into the left anterior descending coronary artery (LAD) with superimposed endothelial damage and distal high-grade stenosis. Intravenous infusion of saline (vehicle), HI (0.3 mg/kg followed by 0.3 mg/kg per hour, 1 mg/kg followed by 1 mg/kg per hour, or 2 mg/kg followed by 2 mg/kg per hour), or HE (60 units/kg followed by 40 units/kg per hour or 100 units/kg followed by 60 units/kg per hour) was initiated 15 minutes before streptokinase (750,000 units for 60 minutes) administration. Vessel patency was monitored for 180 minutes after streptokinase administration with a volume flow probe on the proximal LAD. In dogs treated with no adjunctive agent (saline control), none of the vessels were recanalized with streptokinase. Both HI and HE promoted reperfusion, inhibited reocclusion, and reduced the residual thrombus mass in a dose-dependent fashion. However, at comparable levels of therapeutic anticoagulation (activated partial thromboplastin time [APTT] = 1.5–2.0 times baseline) HI exhibited a higher incidence of reperfusion (eight of eight dogs [100%] versus one of eight dogs [12%]), a shorter time to reperfusion (33±6 versus 59 minutes), a longer duration of initial reperfusion (106±21 versus 10 minutes), and a smaller residual thrombus mass than did HE. Likewise, the slope of the relation between the APTT prolongation and the total reperfusion time (“anticoagulation/thrombosis profile”) was almost five times higher for the combined HI data than for the HE data. Our results indicate that HI is more effective than HE in enhancing and sustaining coronary recanalization with streptokinase at a HI dose that modestly prolongs coagulation time and does not alter bleeding times.

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KEY WORDS • thrombolysis • thrombosis • streptokinase • thrombin inhibition • anticoagulation • hirudin • heparin

Resistance to coronary recanalization and clot lysis with subsequent acute thrombotic recocclusion continue to limit the effectiveness of thrombolytic therapy even in the presence of adjunctive antiplatelet and anticoagulant agents such as aspirin and heparin (HE). The antithrombotic efficacy of HE may be limited primarily because it is an indirect inhibitor of thrombin requiring antithrombin III but also because HE may be neutralized by substances released by platelets and may itself stimulate platelets. For these reasons and because of the potent activation of platelets by thrombin, direct inhibitors of thrombin appear to exhibit a distinct antithrombotic advantage over the indirectly acting anticoagulant HE.

A naturally occurring, potent, and specific thrombin inhibitor was first isolated from the salivary glands of the medicinal leech (Hirudo medicinalis) in the late 1950s. The substance, termed “hirudin,” was later purified and characterized as a 65–amino acid polypeptide. Laboratory investigation of hirudin, especially in vivo studies, was limited by the lack of availability of adequate quantities of the pure peptide. However, this problem has been overcome by the production, through recombinant DNA technology, of several variants of the natural hirudin.

Hirudin is presently being developed as a venous and arterial antithrombotic agent. Most of the in vivo preclinical studies with hirudin have involved the prevention of systemic venous or arterial thrombi. Limited animal studies have been reported comparing the efficacy of hirudin and HE as adjuncts to coronary thrombolysis. All of these studies have been restricted to using hirudin as an adjunct to the thrombolytic agent tissue-type plasminogen activator (t-PA). Recent clinical trials demonstrating comparable thrombolytic efficacy and bleeding complications associated with t-PA and streptokinase, combined with the substantially lower cost of streptokinase, have yielded a renewed clinical interest in streptokinase. Therefore, the purpose of this study was to compare the dose–response relations between recombinant desulfatohirudin (HI) and HE as adjuncts to streptokinase thrombolysis in a canine model of coronary arterial thrombosis.

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Materials and Methods

Animal Preparation

Experiments were conducted on colony-bred male mongrel dogs weighing 17–24 kg (Barton’s West End Farms, Oxford, N.J.). All potential experimental animals were screened for normal activated partial thromboplastin times (APTTs) and prothrombin times (PTs) while in the conscious state upon entry to our colony. Coagulation times were measured on 36 dogs to determine a normal range for our population. The resulting normal ranges for APTT and PT were 8.2–16.1 [(12.16±3.92 (mean±2 SD)] seconds and 6.2–7.2 (6.72±0.52) seconds, respectively. Only dogs with APTTs and PTs within these respective ranges were used for the subsequent coronary thrombosis experiments. All animal studies and procedures were approved by the institutional animal care committee and conducted in accordance with the guidelines established in “Guide for the Care and Use of Laboratory Animals” (US Department of Health and Human Services publication No. [NIH] 86-23).

On the day of the thrombosis study, dogs were anesthetized with pentobarbital sodium (30 mg/kg i.v.). A stable plane of anesthesia was maintained throughout the experiment by administering supplemental doses of intravenous pentobarbital. Rectal temperature was monitored and controlled within ±0.5°C of baseline temperature with a thermal blanket and heat lamp.

After intubation with auffed endotracheal tube, the animals were ventilated with room air by means of a positive-pressure respirator (model 607, Harvard Apparatus, South Natick, Mass.). The volume was adjusted to 15 ml/kg body weight. A positive end-expiratory pressure of 5–7 cm water was applied to prevent atelectasis. Arterial blood gases and pH were measured frequently (model ABL-3, Radiometer America, Inc., Westlake, Ohio) and maintained within a physiological range (pH 7.35–7.45; PCO2, 30–40 mm Hg; PO2>80 mm Hg) by adjusting the respiratory rate, administering sodium bicarbonate, and supplementing the inspired gas with 100% O2 as required.

Cannulas were inserted into a femoral vein for injection and infusion of drugs, pentobarbital, and bicarbonate. Systemic arterial pressure was measured with a pressure transducer (model P23XL, Gould Instruments, Cleveland, Ohio) and a catheter that was inserted into the aorta via a femoral artery. Mean arterial pressure was derived by electronic integration of the pulsatile pressure signal.

The heart was exposed through a thoracotomy at the left fifth intercostal space. The pericardial sac was opened, and a pericardial cradle was created. Left ventricular pressure was measured by means of a pressure transducer cannula (Mikro-tip, model MPC 500, Millar Instruments, Inc., Houston, Texas) inserted into the left ventricular chamber through an apical stab wound. The first derivative of the left ventricular pressure was derived by electronically differentiating the pressure signal. Heart rate was obtained by triggering a pressure processor amplifier (model 13-4615-52, Gould) with the left ventricular pressure signal.

The coronary thrombosis model used in this study was implemented according to the procedure first described by Gold et al.16 and later modified by the same group to include a distal high-grade coronary stenosis.17 A 2-cm segment of the proximal left anterior descending coronary artery (LAD) was isolated. All minor branches of the artery were ligated. A cannula (PE-20) was inserted into one of the branches located in the midportion of the isolated segment. The arterial segment was instrumented from the distal to the proximal end with a plastic screw clamp, a ligature snare, the side-branch cannula, a second ligature snare, and a transit time ultrasonic flow probe (model 2RB probe, model T201 meter, Transonic Systems, Inc., Ithaca, N.Y.). A recording electrode was implanted in the subendocardium of the left ventricular region perfused by the distal LAD for monitoring the ST segment on the electrogram.

Left ventricular pressure and its first derivative, LAD flow, arterial pressure, and the electrogram signals were displayed on a strip-chart recorder (model 3800, Gould). Instantaneous heart rate was monitored on a digital display (model 53-G2682-09, Gould).

Lidocaine was infused prophylactically (0.1 mg/kg per minute intravenously) for the duration of the study to minimize arrhythmias consequent to LAD occlusion and reperfusion. After stabilization of hemodynamic, respiratory, and metabolic parameters, baseline LAD flow was recorded. By adjusting the screw clamp, LAD flow was reduced to ~40% of baseline flow. Developers of this model have determined angiographically that this degree of flow restriction yields a ≥90% reduction in the luminal diameter.17 To promote thrombus adherence to the vessel wall, the endotherhielum of the LAD in the region between the ligature snares was traumatized by four or five compressions of the artery with a hemostat. Both ligature snares were closed to form a blind sac. An occlusive thrombus was formed by injecting a mixture of fresh autologous whole blood (0.3 ml) and thrombin (10-40 units) into the isolated LAD via the cannulated side branch. Five minutes and 8 minutes later, the proximal and distal ligature snares, respectively, were released.

Experimental Protocol

The experimental protocol (Figure 1) was initiated after allowing the occlusive thrombus to age for at least 30 minutes. Adjunctive drug therapy was then administered and continued for the duration of the protocol (Figure 1). Drugs consisted of either 0.9% saline control, one of three doses of HI (0.3 mg/kg per hour, 1 mg/kg followed by 1 mg/kg per hour, or 2 mg/kg followed by 2 mg/kg per hour), or one of two doses of HE (60 units/kg followed by 40 units/kg per hour or 100 units/kg followed by 60 units/kg per hour). Fifteen minutes after beginning adjunctive drug infusion, streptokinase (750,000 IU) was initiated and infused over the ensuing 60 minutes (Figure 1).

Vessel patency was monitored for 180 minutes after streptokinase infusion with the flow probe on the LAD. “Reperfusion” after thrombolysis was defined as a return of LAD flow to >50% of the baseline (after stenosis) flow for a period of at least 5 minutes. A subsequent vessel “reoclusion” was documented when flow persisted at <50% of baseline for at least 5 minutes. Because some dogs exhibited cyclical flow variations, subsequent reperfusion/reoclusion patterns were defined by the same criteria as the original reperfusion/reoclusion. Parameters evaluated within each
treatment group were 1) time to reperfusion (i.e., time after initiating streptokinase until documented reperfusion, only for vessels that were recanalized), 2) incidence of reperfusion, 3) time to reocclusion (i.e., time from a documented reperfusion until a documented reocclusion, only for vessels that reoccluded), 4) incidence of reocclusion, 5) duration of initial reperfusion (i.e., time from a documented reperfusion until either a documented reocclusion or experiment terminus, for all vessels that were reperfused), and 6) total duration of reperfusion (summation of the time in which LAD flow was ≥50% of baseline independent of the duration of reflow, only for vessels with documented reperfusion).

Hemodynamic parameters (left ventricular pressure [systolic and end diastolic] and its first derivative, mean arterial pressure, and heart rate) were measured before thrombus formation (after stenosis), before and 15 minutes after initiating adjunctive drug therapy, and at 30, 60, 120, and 180 minutes after initiating streptokinase infusion (Figure 1).

Arterial blood samples (Figure 1) were also collected coincident with each hemodynamic measurement (except for the prethrombus time point) for later determination of APTT and PT. Blood was withdrawn and anticoagulated with 1/10th vol buffered trisodium citrate (3.8%, Vacutainer, Becton Dickinson). Platelet-free plasma was obtained by centrifuging the blood for 10 minutes at 3,000g. Prepared plasma samples were stored at −20°C until the time of processing. Coagulation times were assessed with an automated coagulation timer (Coag-A-Mate X2, Organon Teknika Corp., Durham, N.C.) and commercial reagents (APTT: Automated APTT, Organon Teknika; PT: Simplastin Excel, Organon Teknika).

Buccal mucosa bleeding times (BMBTs) were measured in triplicate before adjunctive drug therapy and at the termination of the experiment according to the procedure of Jergens et al.18 Briefly, venous return to the upper lip was retarded by tying a gauze strip around the maxilla. A 1-mm-deep and 5-mm-long cut was created by activating a spring-loaded scalpel device (Surgicutt, International Technidyne Corp., Edison, N.J.) over the selected mucosal site. Shed blood was blotted with filter paper until bleeding stopped. Also, at the experiment terminus, the residual thrombus was carefully removed from the LAD and weighed.

Drugs

HI (CGP 39393) used in this study was produced in yeast (Saccharomyces cerevisiae) and purified to homogeneity as described earlier.19 This peptide has the same amino acid sequence as the HV-1 variant of the natural hirudin but lacks the sulfate on the tyrosine 63 residue. All experiments were conducted with a single lot of HI. HE (standard porcine intestinal heparin, Elkins-Sinn, Inc., Cherry Hill, N.J.), HI, lidocaine HCl (LyphoMed, Inc., Rosemont, Ill.), and streptokinase (Streptase, 750,000 IU, Astra Pharmaceutical Products, Inc., Westborough, Mass.) were prepared in 0.9% saline and infused at a rate of 0.15 ml/min (HE and HI) or 0.4 ml/min (lidocaine and streptokinase). Thrombin (bovine origin, Thrombostat, Parke-Davis, Morris Plains, N.J.) was dissolved in 0.9% saline to achieve a concentration of 100 units/ml, aliquotted, and frozen until used. All other drugs were prepared on the day of the experiment.

Statistical Analyses

Time-weighted averages of APTT and PT values for each dog were determined by trapezoidal integration over time. APTT, PT, and BMBT baselines and changes in these parameters (i.e., time-weighted averages as a multiple of control) as well as the thrombus masses were
compared by one-way analysis of variance (ANOVA) and Scheffe’s multiple comparisons.

Linear regression analysis was applied to the APTT versus total reperfusion time values or versus the residual thrombus mass for the combined data within the HI- and HE-treated groups to determine the slopes of these relations. Significance within each relation was determined by an F test. Analysis of covariance with APTT as the covariate was used to discern differences between the slopes for the relations.

Incidence of reperfusion and incidence of reocclusion were compared between the HI- and HE-treated groups with a two-tailed Fisher’s exact test. Times to reperfusion were compared with standard survival analysis and the Mantel-Haenszel log-rank test on all eight dogs within each of the five drug-treated groups. Duration of initial reperfusion was analyzed in a similar manner but only on those dogs in which the vessel reperfused. Differences in time to reocclusion and the total duration of reperfusion were not analyzed because of the low number of dogs reoccluding in each group and because a summation of multiple flow cycles was not appropriate for survival analysis, respectively.

Each hemodynamic parameter within each treatment group was analyzed over time with a repeated-measures one-way ANOVA and Dunnett’s comparison with the baseline (after stenosis) value.

In all analyses, a value of $p \leq 0.05$ was considered statistically significant. Data are presented as mean ± SEM.

**Results**

Figure 2 displays the times of documented reperfusion for each of the eight dogs within each drug regimen. There was no reperfusion in any of the saline-treated dogs, so these data are excluded from the figure. Streptokinase was initiated at time 0 and continued for 60 minutes. Hirudin (0.3 mg/kg followed by 0.3 mg/kg per hour, 1.0 mg/kg followed by 1.0 mg/kg per hour, or 2.0 mg/kg followed by 2.0 mg/kg per hour) and heparin (60 units/kg followed by 40 units/kg per hour or 100 units/kg followed by 60 units/kg per hour) were administered 15 minutes before streptokinase infusion.
The various reperfusion/reocclusion parameters from the individual experiments in Figure 2 are summarized in Table 1. Numbers in parentheses indicate the number of animals corresponding to each data value. There was a dose-dependent increase in the incidence of reperfusion for both HI and HE when administered as adjuncts to streptokinase. The high and medium doses of HI resulted in 100% and 75% incidences of reperfusion, respectively. These values were not significantly different from the results with the high dose of HE (75%) but were statistically greater than those with the low dose of HE. At this dose of HE, only one of the eight dogs experienced coronary recanalization with streptokinase.

Although the incidence of reocclusion decreased from 50% to 33% with the first two doses of HI, there was no additional benefit with the highest dose. Likewise, the duration of the initial reperfusion period and the total time of reperfusion doubled from the low to medium dose of HI but did not increase further with the highest dose. With HE, the incidence of reocclusion was high (100% and 83%) with either dose. Although the incidence of reocclusion tended to be lower with HI than with HE, this difference did not reach statistical significance, probably because of the low number of animals exhibiting reperfusion within some of the groups (e.g., the HE group infused with 60 units/kg followed by 40 units/kg per hour).

Comparison of the two groups indicates that the time to reperfusion was reduced, and the duration of the initial reperfusion was prolonged in the high-dose HI group to a greater extent than the corresponding parameters in the high-dose HE group. Likewise, with respect to the same two parameters, the medium dose of HI was superior to the low dose of HE. Thus, whereas the highest dose of HI yielded a rapid (33 minutes) and sustained (106 minutes) recanalization after streptokinase, vessels treated with the high dose of HE experienced only a brief period of reflow (46 minutes) after a delayed onset of 65 minutes.

APTT and PT were measured throughout the experimental protocol as an index of the degree of anticoagulation. APTT and PT (both as a multiple of control) versus time for each drug regimen are displayed in Figure 3. Both agents caused a dose-dependent increase in each coagulation time. After an initial peak, each parameter quickly reached a steady state and was maintained throughout the duration of the protocol.

Table 2 depicts the control (in seconds) and change (as a multiple of control) in the APTT, PT, and the BMBT for the six groups. There were no differences in baseline APTT, PT, or BMBT between the six groups (Table 2). APTT and PT were unchanged in the saline-treated dogs (Table 2). APTT and PT increased significantly in a dose-dependent fashion within the HI and HE groups. Differences between groups are indicated in the table. Briefly, the high dose of HE prolonged APTT significantly greater than did each dose of HI, whereas the response to the low dose of HE exceeded that of only the low dose of HI. Although BMBTs increased by 20–30% in the saline and five drug groups, these changes were not significantly different between the six groups.

Comparison of the change in APTT versus the total reperfusion time or versus residual thrombus mass for the HI and HE groups reflected dramatically different anticoagulant/antithrombosis profiles for the two agents (Figure 4). Whereas nearly maximal reperfusion (180 minutes minus a finite time for the onset of reperfusion) occurred with HI at an APTT increase in the low therapeutic range (1.5–2.0), the threshold for reperfusion in the HE group was at an APTT increase of 1.8 times control. Likewise, all of the HE-treated dogs that exhibited significant recanalization had APTT increases outside the therapeutic range (i.e., >2.5 times control). Linear regression analysis was applied to the APTT versus total reperfusion time relations for the combined data within the HI and HE groups (Figure 4, top panel). The resulting estimated slopes were 92 and 20 for the HI and HE data, respectively. These slopes were statistically different, as indicated by the significant interaction effect from analysis of covariance.

Likewise, there was a significantly greater reduction in residual thrombus mass with HI than with HE for similar degrees of anticoagulation (i.e., increase in APTT) (Figure 4, bottom panel). Thus, each group exhibited drug- and dose-dependent decreases in residual thrombus mass that were coincident with the incidence of vessel recanalization (Figure 5). In the saline-

<table>
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<tr>
<th>Table 1. Effects of Hirudin (CGP 39393) and Heparin in Conjunction with Streptokinase on Reperfusion and Reocclusion Parameters in Anesthetized Dogs</th>
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<tr>
<td>HI 0.3</td>
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<td>HI 2</td>
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<tr>
<td>HE 60</td>
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<td>HE 100</td>
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HI, hirudin; HE, heparin; HI 0.3; 0.3 mg/kg followed by 0.3 mg/kg per hour; HI 1, 1 mg/kg followed by 1 mg/kg per hour; HI 2, 2 mg/kg followed by 2 mg/kg per hour; HE 60, 60 units/kg followed by 40 units/kg per hour; HE 100, 100 units/kg followed by 60 units/kg per hour.

Incidence values indicate the percentage (fraction) of animals responding to a particular treatment. All time values are mean±SEM. Numbers in parentheses reflect the number of animals corresponding to each data value. HI and HE were administered 15 minutes before streptokinase (750,000 units for 1 hour).

*P<0.05 vs. HE 60 value.

†P<0.05 vs. HE 100 value.
treated dogs, thrombus mass was consistently 25–35 mg (Figure 5). All doses of both HI and HE significantly decreased the thrombus masses when compared with the saline-treated group (Figure 5). The high dose of HI exhibited the most dramatic and consistent decrease in residual thrombus mass (0.4–1.2 mg). These thrombi were significantly smaller than those in the low-dose HI and low-dose HE groups but did not reach statistical significance when compared with the high dose of HE, probably because of the variability within the latter group (Figure 5). Doses of HI and HE that only slightly prolonged APTT (i.e., increase of ~1.2 times control) caused similar reductions in thrombus mass from ~30 mg with streptokinase alone (i.e., saline treated) to ~10 mg with the adjunctive agents (Figure 4, bottom panel). However, further increases in APTT by HE yielded little additional benefit in reducing thrombus mass. On the contrary, increasing APTT to 2.5 times control with HI further reduced residual thrombus mass by ~30-fold (Figure 4, bottom panel). These differences are re-

| Table 2. Effects of Saline, Hirudin, and Heparin in Conjunction With Streptokinase on Coagulation and Bleeding Times in Anesthetized Dogs |

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<tr>
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<th>APTT</th>
<th>PT</th>
<th>BMBT</th>
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<tr>
<td></td>
<td>Control (seconds)</td>
<td>Δ(×control)</td>
<td>Control (seconds)</td>
</tr>
<tr>
<td>Saline</td>
<td>12.0±0.3</td>
<td>...</td>
<td>6.8±0.1</td>
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<tr>
<td>HI 0.3</td>
<td>11.8±0.5</td>
<td>1.22±0.03 *†</td>
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<td>1.54±0.03 *†</td>
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<td>3.34±0.21</td>
<td>6.7±0.1</td>
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APTT, activated partial thromboplastin time; PT, prothrombin time; BMBT, buccal mucosa bleeding time; HI, hirudin; HE, heparin; HI 0.3; 0.3 mg/kg followed by 0.3 mg/kg per hour; HI 1, 1 mg/kg followed by 1 mg/kg per hour; HI 2, 2 mg/kg followed by 2 mg/kg per hour; HE 60, 60 units/kg followed by 40 units/kg per hour; HE 100, 100 units/kg followed by 60 units/kg per hour. Values are mean±SEM; n=8 for all six groups.

APTT, PT, and BMBT are presented as control and change as a multiple of control (×control). Changes in APTT and PT are time-weighted averages over the course of the experimental protocol. BMBTs were measured in triplicate before initiating saline, HI, or HE and at the termination of the experiment. Saline, HI, and HE were administered 15 minutes before streptokinase (750,000 units for 1 hour). *p<0.05 vs HI 60 value. †p<0.05 vs. HE 100 value.
It should be noted that in one of the dogs in the high-dose HI group the APTT and PT readings were unmeasurable (i.e., >150 seconds) after HI infusion. Because the responsiveness to HI in this dog was clearly an outlier, the APTT and PT values are not included in Figures 3 and 4 and in Table 2.

Hemodynamic parameters at various times throughout the protocol for the six groups of dogs are displayed in Table 3. Changes were minor in all cases except for left ventricular dP/dt, which decreased by 25–30% in all six groups by the final 1–2 hours of the protocol. Thus, there appear to be either no substantial hemodynamic changes or consistent drug-independent hemodynamic changes between groups.

Discussion

Thrombin plays a key role in the bioregulation of hemostasis and thrombosis. As the final common mediator of the intrinsic and extrinsic coagulation pathways, thrombin proteolytically cleaves fibrinogen to form the fibrin monomer. Feedback activation of factors V and VIII by thrombin amplifies its own generation by producing prothrombinase complexes that then cleave prothrombin to yield additional thrombin. Fibrin polymerization forms a matrix that is stabilized by thrombin’s activation of factor XIII. This stabilization process renders the clot insoluble and increases its resistance to subsequent fibrinolysis. However, clot dissolution itself can enhance thrombin generation by platelet-dependent mechanisms or by releasing enzymatically active thrombin bound to fibrin within the clot.21–24 The released or reexposed thrombin can lead to further deposition of platelets onto a preformed thrombus since thrombin is a potent platelet activator. Thus, based on thrombin’s procoagulant and platelet proaggregatory effects, inhibition of this protease is an attractive pharmacological approach to antithrombotic.

HE, an indirect thrombin inhibitor, has been used clinically and experimentally as a pharmacological adjunct to thrombolysis. However, recent clinical and animal studies indicate that HE may have limited ability to promote coronary thrombolysis or to prevent rethrombosis after successful thrombolysis.11,14,15,25–27 The limited effectiveness of HE was corroborated in our animal model of coronary arterial thrombosis. At a dose of HE that yielded a therapeutic prolongation of the APTT, the incidence of coronary recanalization after streptokinase infusion was enhanced to only 12% (one of eight vessels) compared with 0% without adjunctive treatment. Subsequently, the one artery that did reperfuse remained patent for only 10 minutes before recloaking. Higher, supratherapeutic doses of HE further enhanced the recanalization rate but maintained the coronary perfusion in a low percentage of animals and for only short durations.

Because of the limitations of the indirect thrombin inhibitor HE and the potent activation of platelets by thrombin, direct thrombin inhibitors would appear to exhibit a distinct antithrombotic advantage over HE. In recent years, considerable effort has been directed toward developing low molecular weight thrombin inhibitors. By virtue of its formation of tight-binding equimolar complexes with thrombin, hirudin is the most potent and specific thrombin inhibitor known. Natural hirudin, a 65-residue peptide, was originally isolated from the periphraryngeal glands of the medicinal leech.
but is now readily available through recombinant technology. In the present study, we used recombinant desulfatorhirudin (HI), which differs from the natural peptide by lacking the sulfate on the Tyr residue. Although this modification yields a slightly lower affinity for thrombin, HI still maintains an extremely high potency and selectivity for thrombin compared with other serine proteases. Additional thrombin inhibitors that are analogues or fragments of the natural hirudin or shorter peptide or peptidelike agents have also been described.

The lower molecular weight of these agents compared with the relatively bulky heparin-antithrombin III complex would be expected to enhance their accessibility to clot-bound thrombin. Indeed, recent studies have shown that, in contrast to HE, hirudin and other direct thrombin inhibitors inactivate both free and fibrin-bound thrombin to approximately the same degree. Hirudin may possess other advantages over HE in that it does not have any known endogenous inactivators, and it inhibits thrombin-induced platelet aggregation and release of potent vasoconstrictor and platelet activating agents from platelets and endothelial cells.

Prior studies with direct thrombin inhibitors have documented the potential benefit of this class of agents as antithrombotics and adjuncts to thrombolysis. For example, in several baboon models of thrombus generation, hirugen, the dedcapeptide fragment of hirudin, interrupted fibrin-dependent thrombosis. Hirugen also promoted clot lysis with t-PA and delayed coronary reclosure in the canine copper coil model of coronary thrombosis. Likewise, the substituted arginine derivative argatroban inhibited thrombus formation and enhanced thrombolysis with t-PA in a variety of canine arterial thrombosis models. Hirudin's efficacy in promoting lysis of arterial thrombi with t-PA has also recently been demonstrated.

Our study demonstrates a dose-dependent enhancement by HI of streptokinase-mediated coronary thrombolysis. These beneficial effects were manifested by an increase in the incidence of and time to reperfusion and a higher incidence and prolongation of coronary patency after successful recanalization. Also, at the highest dose of HI, residual thrombus mass was reduced by 40-fold compared with streptokinase alone, indicating that direct thrombin inhibition not only enhances clot lysis but also limits further clot extension after terminating fibrinolytic therapy. HE also moderately enhanced coronary recanalization and reduced thrombus mass with streptokinase in a dose-dependent manner but did not accelerate clot lysis. These results probably reflect the limited ability of HE to inhibit preformed thrombin bound to the original fibrin-rich clot in our thrombosis model. The nearly 100% incidence of reclosure may also be indicative of HE's ineffectiveness in inhibiting thrombin-stimulated platelet deposition onto a lysed and reformed clot, which in this model has been shown to be platelet rich. On the other hand, HI's ability to limit clot extension in our model suggests that significant inhibition of platelet aggregation was achieved at the utilized doses. Similar profiles by hirudin and HE in a canine model of electrically induced platelet-rich coronary thrombus formation support this contention. Haskel et al. reported that in conscious dogs t-PA alone lysed all of the (platelet-rich) thrombi but that after discontinuing lytic therapy occlusive thrombi reformed in each case. A single dose of HI (1.5 mg/kg followed by 1.5 mg/kg per hour), which prolonged APTT by 1.7-fold, shortened the recanalization time and completely prevented reclosure, whereas HE at a dose that comparably increased coagulation times had no beneficial effect on these parameters. Although the overall incidence of recanalization was lower because of the presence of a critical coronary stenosis, a similar separation between the adjunctive effects of hirudin or HE combined with t-PA was also reported in this model in anesthetized dogs. Recent studies with carotid angioplasty in anesthetized pigs further document HI's ability to inhibit platelet deposition at the site of arterial injury. Whereas HI inhibited platelet and fibrinogen deposition to the injured wall and completely prevented mural thrombosis,
TABLE 3. Effects of Saline, Hirudin, and Heparin in Conjunction with Streptokinase on Hemodynamic Parameters in Anesthetized Dogs

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (after stenosis)</th>
<th>Before drug (after thrombus)</th>
<th>After initiation of streptokinase infusion (minutes)</th>
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<tr>
<td>LVSP (mm Hg)</td>
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<td>122±3</td>
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<tr>
<td>HI 1</td>
<td>121±2</td>
<td>121±2</td>
<td>120±4</td>
</tr>
<tr>
<td>HI 2</td>
<td>119±5</td>
<td>121±6</td>
<td>124±5</td>
</tr>
<tr>
<td>HE 60</td>
<td>126±5</td>
<td>117±5</td>
<td>119±4</td>
</tr>
<tr>
<td>HE 100</td>
<td>124±3</td>
<td>118±4</td>
<td>122±4</td>
</tr>
</tbody>
</table>

| LVEDP (mm Hg)       |                           | 11±1 | 12±1 | 12±1 | 12±1 | 11±1 | 11±1 |       |
| Saline              | 10±1                      | 11±1 |      |      |      |      |      |       |
| HI 0.3              | 10±1                      | 12±1 | 12±1 | 12±1 | 11±1 | 11±1 |       |
| HI 1                | 9±1                       | 10±1 | 11±1 | 10±1 | 10±1 | 10±1 |       |
| HI 2                | 11±1                      | 13±1 | 14±1 | 13±1 | 14±1 | 12±1 |       |
| HE 60               | 11±1                      | 13±2 | 13±2 | 13±1 | 13±1 | 12±1 |       |
| HE 100              | 9±1                       | 10±2 | 10±1 | 10±1 | 11±1 | 10±2 |       |

| LV dP/dt (mm Hg/sec) |                           | 1,825±78 | 1,800±86 | 1,863±101 | 1,569±102 | 1,500±121 | 1,319±89 | 1,288±77 |
| Saline              | 1,825±78                  |          |          |          |          |          |          |          |
| HI 0.3              | 2,025±124                 | 1,844±133 | 1,981±168 | 1,847±139 | 1,794±119 | 1,556±89 | 1,456±102 |
| HI 1                | 2,125±97                  | 2,069±107 | 1,983±138 | 1,938±142 | 1,853±129 | 1,790±122 | 1,756±80 |
| HI 2                | 1,713±71                  | 1,663±78 | 1,663±75 | 1,594±80 | 1,581±51 | 1,356±74 | 1,300±112 |
| HE 60               | 1,863±131                 | 1,711±148 | 1,770±121 | 1,733±101 | 1,663±114 | 1,543±144 | 1,358±119 |
| HE 100              | 1,914±47                  | 1,800±49 | 1,836±50 | 1,807±85 | 1,843±74 | 1,629±69 | 1,471±36 |

| MAP (mm Hg)         |                           | 116±5 | 119±2 | 121±2 | 115±3 | 108±3 | 104±5 | 101±5 |
| Saline              | 116±5                     |          |          |          |          |          |          |          |
| HI 0.3              | 117±3                     | 114±3 | 123±3 | 119±4 | 115±2 | 113±3 | 109±5 |
| HI 1                | 114±5                     | 113±3 | 113±3 | 113±3 | 113±3 | 110±2 |
| HI 2                | 113±5                     | 115±6 | 120±4 | 116±5 | 114±5 | 105±6 | 104±7 |
| HE 60               | 118±5                     | 113±4 | 113±4 | 112±5 | 110±4 | 102±6 | 94±6 |
| HE 100              | 119±3                     | 113±4 | 118±4 | 117±3 | 120±2 | 111±6 | 109±6 |

| HR (bpm)            |                           | 150±7 | 156±6 | 161±6 | 147±7 | 148±6 | 153±7 | 157±7 |
| Saline              | 150±7                     |          |          |          |          |          |          |          |
| HI 0.3              | 152±7                     | 149±7 | 153±8 | 148±6 | 144±5 | 152±7 | 163±4 |
| HI 1                | 162±5                     | 161±6 | 157±8 | 157±8 | 160±6 | 164±6 | 169±5 |
| HI 2                | 141±5                     | 142±5 | 144±5 | 143±4 | 147±5 | 149±4 | 156±4 |
| HE 60               | 150±4                     | 160±5 | 160±5 | 165±4* | 165±4* | 169±5 | 168±5* |
| HE 100              | 149±7                     | 148±7 | 149±7 | 150±7 | 151±8 | 158±7 | 166±6 |

LVSP, left ventricular (LV) systolic pressure; HI, hirudin; HE, heparin; HI 0.3; 0.3 mg/kg followed by 0.3 mg/kg per hour; HI 1, 1 mg/kg followed by 1 mg/kg per hour; HI 2, 2 mg/kg followed by 2 mg/kg per hour; HE 60, 60 units/kg followed by 40 units/kg per hour; HE 100, 100 units/kg followed by 60 units/kg per hour. LVEDP, LV end-diastolic pressure; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute. Values are mean±SEM (n=8 for all six groups).

LVSP, LVEDP and its first derivative (LV dP/dt), MAP, and HR were measured at the indicated times. Baseline values were determined after applying a stenosis to the left anterior descending coronary artery. Predrug values were measured after allowing an occlusive left anterior descending coronary artery thrombus to incubate for 30 minutes. Saline, HI, and HE were administered 15 minutes before streptokinase (750,000 units for 1 hour).

*p<0.05 vs. baseline data.

therapeutic doses of HE and antiplatelet agents exhibited either no benefit or only modest effects in this porcine angioplasty model.

The antithrombotic advantages of HI over HE in our study are illustrated by the disparate anticoagulant/antithrombosis profiles of the two agents (Figure 4). These relations indicate that a particular prolongation of APTT with anticoagulant therapy is itself a poor predictor of antithrombotic potential. However, within either agent, the APTT was a reliable predictor of the corresponding antithrombotic efficacy. The difference in slopes between these relations indicates that for a particular prolongation of APTT, HI yielded approximately five times greater reperfusion than did HE. Thus, although nearly maximal reperfusion occurred with HI at an APTT increase in the low therapeutic range (1.5–2.0), the threshold for reperfusion in the HE group was at an APTT increase of 1.8 times control. Likewise, all of the HE-treated dogs that exhibited significant recanalization had APTT increases outside the therapeutic range (i.e., >2.5 times control). Supratherapeutic APTT prolongation by HE in other animal models has been reported to not prevent reocclusion after thrombolysis,

\[14,49,50\]
HI was also an order of magnitude more effective than HE in reducing residual thrombus mass at a comparable therapeutic level of anticoagulation (Figure 4, bottom panel). In addition, for a given residual thrombus mass there was a greater degree of reperfusion in the HI- than in the HE-treated vessels. This is evident from the results in Figure 5 in which no reperfusion occurred in vessels from either treatment group with thrombus masses above \( \approx 8-9 \, \text{mg} \). All of the vessels in the HI group with thrombus masses below this apparent reperfusion threshold exhibited significant reflow (open circles in Figure 5). On the contrary, \( \approx 40\% \) of the HE-treated vessels with thrombi below this level remained resistant to recanalization by streptokinase. Also, of those that did exhibit reperfusion, the total reperfusion time was short because of the high incidence of reclosure or cyclic flow variations in the HE groups. These observations suggest that other factors, in addition to the absolute reduction in thrombus mass, may interact to determine reflow in these vessels. For example, HE may directly stimulate platelets and increase local platelet production of thromboxane A\(_2\), whereas HI has been demonstrated to inhibit platelets and the release of potent vasoconstrictor agents.\textsuperscript{46–48,60,61} This may be of added importance during t-PA or streptokinase therapy in which HE, but not HI, prevents the reduced platelet aggregatory response to plasmin generation.\textsuperscript{62,63} Thus, adjunctive thrombolytic treatment with HE may promote vasospastic events and thereby elevate the threshold for functional recanalization by further constricting the underlying stenosis.\textsuperscript{64,65}

Collectively, our findings are consistent with clinical observations that an unacceptable percentage of patients treated with HE at therapeutic levels of anticoagulation remain resistant to coronary clot dissolution by fibrinolytics or experience an undesirably high incidence of subsequent reclosure.\textsuperscript{26,27} Our results with streptokinase combined with the favorable results by others with t-PA in various animal models suggest that HI at moderate levels of anticoagulation should exhibit a clear adjunctive thrombolytic advantage over HE in patients treated for acute myocardial infarction.

Hirudin is well tolerated clinically and in laboratory animals.\textsuperscript{66–69} In dogs, acute administration of 10 mg/kg hirudin (five times our highest dose) caused no hemodynamic effects.\textsuperscript{68} Template bleeding times in humans are not significantly prolonged after therapeutic intravenous doses of hirudin.\textsuperscript{38,69} Likewise, in various animal models, bleeding complications are detected only at relatively high doses of hirudin that are at least an order of magnitude greater than the doses required for significant antithrombosis.\textsuperscript{70,71} When administered in conjunction with streptokinase (present study) or t-PA,\textsuperscript{11} HI (i.e., CGP 39393) also does not significantly prolong template bleeding times in dogs. Recent studies have revealed that the prolongation of template bleeding time is a reliable predictor of hemorrhagic diathesis occurring in animals and patients treated with t-PA and aspirin.\textsuperscript{72,73} Therefore, HI, in contrast to specific antiplatelet agents,\textsuperscript{11,55,74} appears to provide a high degree of antithrombosis without impeding primary hemostasis and predisposing the recipient to bleeding complications. Hirudin also appears to be a weak immunogen, indicating that repeat exposures to the agent are unlikely to elicit immune reactions or to compromise its antithrombolytic efficacy.\textsuperscript{96,97,98,75,76}

In summary, our results demonstrate that in our canine model, HI is a more effective adjunct to streptokinase-mediated thrombolysis than HE at comparable levels of anticoagulation. The dose-dependent prolongation of APTT by either HI or HE appears to be a reliable predictor of the antithrombotic potential within each agent. However, whereas HE exhibits minimal effectiveness even at high therapeutic doses, HI is maximally effective at low to mid therapeutic levels of anticoagulation. Extension of these results to the clinical arena suggests that direct inhibition of thrombin with HI may be an effective approach to enhancing and sustaining coronary thrombolysis with streptokinase without the untoward bleeding complications of various other adjunctive agents.

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References

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34. Kikumoto R, Tamao Y, Tezuka T, Tomonura S, Hara H, Nominaya H, Hikijaka A, Okamoto S: Selective inhibition of thrombin by (2R,4R)-4-methyl-1H-[1,2,3,4-tetrahydro-8-quinoli-
Comparison of hirudin and heparin as adjuncts to streptokinase thrombolysis in a canine model of coronary thrombosis.
D F Rigel, R W Olson and R W Lappe

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