Recombinant Hirudin in Patients With Chronic, Stable Coronary Artery Disease
Safety, Half-life, and Effect on Coagulation Parameters

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Background. Because the specific antithrombin hirudin prevents platelet-rich arterial thrombus and accelerates thrombolysis in a variety of animal models, it has promise as antithrombotic therapy. We therefore studied the half-life, effect on anticoagulant parameters, and safety of hirudin in patients with coronary artery disease.

Methods and Results. Thirty-eight men and 1 woman (age [mean±SD], 60.4±6.9 years) with angiographic coronary disease were allocated in a single-blind ascending dosage study to a 6-hour IV infusion of recombinant hirudin (CGP 39,393) or matching placebo. The median terminal half-life for hirudin, measured by ELISA, was 2.7, 2.3, 2.9, 3.1, and 2.0 hours for the 0.02, 0.05, 0.1, 0.2, and 0.3 mg·kg⁻¹·h⁻¹ groups, respectively. Activated partial thromboplastin times (aPTT) at 3, 4, and 6 hours were averaged into a plateau value. The aPTT plateau-to-baseline ratios were 1.5±0.1, 2.0±0.1, 2.3±0.1, 2.7±0.1, and 2.9±0.1, respectively, with hirudin infused at 0.02, 0.05, 0.1, 0.2, and 0.3 mg·kg⁻¹·h⁻¹. From 62% to 77% of the aPTT plateau value was seen within 30 minutes of starting the infusions and was directly related to dose. The aPTT-to-baseline ratios correlated well with plasma hirudin levels (r=.88), whereas poor correlation and sensitivity were observed between plasma hirudin levels and activated coagulation time (ACT)-to-baseline ratios (r=.44). Plasma levels of hirudin and ACT in seconds correlated overall well (r=.80), but considerable overlap occurred between baseline ACT and ACT at plasma hirudin concentrations <1000 ng/mL. Prothrombin times were significantly prolonged only at a dosage of ≥0.05 mg·kg⁻¹·h⁻¹ and were 11.8±0.5 (INR=1.0), 12.3±0.7 (INR=1.1), 13.3±1.2 (INR=1.4), 14.2±0.4 (INR=1.7), and 15.8±0.9 (INR=2.3) seconds for each respective hirudin dosage. Thrombin times were beyond range (>600 seconds) at 6 hours in all except 2 patients who received the lowest dosage. All parameters returned to baseline between 8 and 18 hours after the infusion. Bleeding times were not significantly prolonged. No side effects occurred. No antibodies to hirudin were detected 2 weeks after the infusion.

Conclusions. Recombinant hirudin has a terminal half-life of 2 to 3 hours. The aPTT correlates well with plasma levels of hirudin and allows close titration over a wide range of anticoagulation, while ACT and prothrombin time are relatively insensitive for monitoring hirudin administration. At anticoagulant levels effective in experimental thrombosis, a 6-hour infusion of hirudin is well tolerated and safe in a predominantly male group of patients with stable coronary atherosclerosis. (Circulation. 1993;88[part 1]:2015-2022.)

KEY WORDS • antithrombotics • thrombolysis • atherosclerosis • hirudin • thrombosis

Platelet-rich thrombus formation at the site of atherosclerotic plaque disruption is the usual cause of acute coronary syndromes and of abrupt arterial closure after percutaneous transluminal angioplasty. Rethrombosis after thrombolysis is based on similar mechanisms but, in addition, results from the high thrombogenicity of the residual thrombus. Because aspirin and heparin have lessened but not eliminated the risk of thrombotic occlusion, novel antithrombotic agents currently under investigation include inhibitors of thrombin, of factor Xa, and of the factor VII–tissue factor complex; antibody fragments to the platelet glycoprotein IIb/IIIa receptor; serotonin and thromboxane A₂ receptor blockers; and recombinant thrombomodulin and activated protein C. Hirudin, a 65-residue anticoagulant peptide from the medicinal leech Hirudo medicinalis, binds thrombin with high specificity and affinity (K₀ for the recombinant analogue 2×10⁻¹⁵ mol/L). It inhibits only thrombin-induced platelet activation, while the response to other agonists remains fully intact. The effectiveness of hirudin as an experimental antithrombotic agent has suggested a pivotal role of thrombin in the pathophysiology of arterial thrombosis. At activated partial thromboplastin times...
boplatin times (aPTTs) of two to three times baseline and without additional antiplatelet drugs, hirudin prevented heparin-resistant, platelet-rich thrombus at the site of carotid angioplasty in the pig.10,23 Substantially reduced (but not completely eliminated) platelet deposition in chronic arteriovenous shunts (baobon) grafted with endarterectomy material.24 and accelerated lysis by recombinant tissue-type plasminogen activator of occlusive platelet-rich thrombus in a pig carotid endarterectomy model13 and in other thrombus models.13,26,27 Thus, hirudin and its congeners are currently the subject of intense clinical investigation as an alternative to heparin for the prevention and treatment of thrombosis and as adjunct to thrombolytic therapy.28-30 We therefore investigated the safety, half-life, and hemostatic effects of five ascending doses of recombinant desulfato hirudin in a group of 39 patients with angiographically documented, clinically stable coronary disease.

Methods

The protocol was approved by the Institutional Review Board of the Mayo Clinic. Recombinant desulfato hirudin (CGP 39 393), hereafter referred to as hirudin, was provided by CIBA-GEIGY Corp, Summit, NJ. Patients were eligible if they had a history of myocardial infarction, unstable angina, or coronary artery bypass grafting (alone or in combination) and if they had undergone within 5 years coronary angiography, revealing at least 50% narrowing of one or more major coronary arteries. Exclusion criteria included a hemoglobin of <11.5 g/dL, serum creatinine >1.5 mg/dL, a history of blood loss, uncontrolled hypertension (180/100), or a recent (<1 year) episode of stroke, unstable angina, or myocardial infarction.

Thirty-eight male and one female patient with stable, angiographically proven coronary disease (age [mean±SD], 60.4±6.9 years) were studied after giving informed consent. At the time of the study, patients had stable class II or no angina. Aspirin, dipyridamole, and other antiplatelet agents were stopped 7 days before the study. None of the patients were on chronic anticoagulant therapy. Cardioactive drugs other than platelet inhibitors were continued as prescribed by each patient’s primary physician. Fourteen patients received a calcium channel blocker, 17 received a β-blocker, and 17 received long-acting nitrates.

Patients were admitted to the Mayo Clinical Research Center the day before administration of hirudin or matched placebo. A complete physical examination, ECG, chest radiograph, routine blood chemistry, complete blood count, and urinalysis were performed. Blood count, blood chemistry, and urinalysis were repeated 24 hours after the hirudin infusion. The baseline serum creatinine of the study patients was 1.0±0.2 mg/dL. An intravenous catheter was placed into a peripheral vein the night before hirudin administration and kept patent by continuous infusion of 0.45% saline solution at 30 mL/h. Hirudin and placebo were administered for 6 hours, and patients were closely monitored during the infusion and for the next 18 hours.

Study Groups

The dosages of hirudin studied were 0.02, 0.05, 0.1, 0.2, and 0.3 mg·kg⁻¹·h⁻¹. In each group, 6 patients received an IV infusion of the drug and 2 placebo patients were given a matched infusion of normal saline, with the exception of the 0.3-mg·kg⁻¹·h⁻¹ group, in which only 1 patient was allocated to placebo. Hirudin, with matching placebo, was administered in ascending dosages. Patients and investigators performing the tests, but not the investigators seeing the patients, were blinded to treatment allocation. Baseline hematologic parameters were obtained at 7:45 AM, followed 15 minutes later by a 6-hour infusion of recombinant hirudin or placebo. No bolus was administered prior to the infusion.

Hemostatic Parameters and Plasma Hirudin Determination

Hemostatic parameters (aPTT, prothrombin time [PT], thrombin time, activated coagulation time [ACT], and bleeding time) and plasma hirudin concentrations were measured at baseline and intermittently for 24 hours. Blood was drawn through an intravenous catheter in the arm contralateral to the hirudin infusion into 3.8% trisodium citrate anticoagulant (vol:vol citrate solution = 9:1). The aPTT was determined at the times plotted (hours:minutes) in Figs 1 through 6 (baseline, 0:15, 0:30, 1:0, 1:30, 2, 3, 4, 6 [stop infusion], 6:0, 6:10, 6:20, 6:30, 6:45, 7:00, 7:30, 8:00, 8:30, 9:00, 10:00, 11:00, 12:00, 14:00, and 24:00). A fully automated photometric clot detection system (MLA Electra 700, Medical Laboratory Automation, Mount Vernon, NY) was used after mixing plasma and CaCl₂ (Ricca Chemical Company, Arlington, Tex) with an activated partial thromboplastin reagent (Organon Teknika, Durham, NC). Controls (American Dade Citrol level 1, 2, and 3) were run daily. The normal aPTT with this method is 26 to 41 seconds for men. When >100 seconds, the aPTT was rechecked using the fibrometer method.

The ACT, a bedside test of global coagulation deficits, was measured at baseline and 3, 4, and 6 hours into the infusion and then intermittently for 4 hours after the infusion. The ACT was determined by the same person with the Hemoconor clot-detecting system (International Technidyne Corp, Edison, NJ) using CAS10 celite-activated (diatomaceous earth) test tubes. Each tube contained 12 mg of celite, a natural coagulation activator, and the manufacturer’s instructions were closely followed. The PT was measured at baseline, at 6 hours (completion of the infusion), and at 24 hours. Thromboplastin-C (International Sensitivity Index, ISI, 2.87, American Dade, Miami, Fla) and CaCl₂ were added to plasma as clotting initiators to obtain measurements of the PT. Clot formation was measured by the optical density using the MLA-700. The normal PT with this method ranges between 10.9 and 12.8 (mean, 11.85) seconds for both men and women.

The thrombin time was determined with bovine thrombin, 1000 units/mL (Thrombostat, Parke-Davis, Morris Plains, NJ), diluted until the clotting time of normal citrated plasma fell in the range of 19.5 to 20.5 seconds. Therefore, 200 μL of the thrombin solution was mixed with 200 μL of plasma of a study subject and a control individual. Tubes were continuously tipped in a 37°C waterbath and clot formation was timed by visual inspection.

Bleeding times were determined by the same person with a single 1-mm-deep and 5-mm-long incision on the
volar surface of the forearm using a fully automated disposable razor blade device (Surgicutt, International Technidyne Corp, Edison, NJ). Thirty seconds before the incision, a sphygmomanometer cuff placed around the upper arm was inflated to 40 mm Hg. The bleeding time was determined on cessation of bleeding while blood was wicked away from the wound site every 30 seconds without directly touching the incision. The sphygmomanometer remained inflated at 40 mm Hg until bleeding ceased. Bleeding times were measured at baseline, 5.5 hours into the infusion, and 18 hours after completion of the infusion.

Plasma hirudin was assayed at the time of aPTT measurement by enzyme-linked immunosorbent assay (ELISA). This ELISA is based on a previously described method, with the modification that purified, murine anti-hirudin monoclonal antibodies (J.M. Schlaeppi, CIBA-GEIGY, Basel, Switzerland) and biotinylated, purified polyclonal sheep anti-hirudin antibodies (G. Stöffler, University of Innsbruck, Austria) were used. After incubation of the plasma samples with these two antibodies, avidin–alkaline phosphatase complex was added. Disodium-p-nitrophenyl phosphate hexahydrate was used as substrate. All assays were performed in triplicate. The assay has a detection limit of 0.8 ng hirudin per milliliter of plasma.

Terminal Half-life
The terminal phase rate constants were determined by the least-squares regression analysis of the terminal log linear phase of the concentration-time profile. The terminal half-life is calculated by the equation $T_{1/2} = 0.693/\text{rate constant}$.

Detection of Antibodies to Hirudin
Blood was drawn 2 weeks after the hirudin and placebo infusion from all patients to screen for the presence of immunoglobulin G (IgG) and IgE antibodies against hirudin, as previously described.

Data Analysis
Results are expressed as mean±SD, with the exception of the aPTT, which was expressed as mean±SEM to allow a composite plot of mean aPTT at all hirudin doses >24 hours. Differences between the hemostatic parameters of the study groups were analyzed with Student's $t$ test or the Wilcoxon rank-sum test. Pearson's correlation coefficient ($r$) was used to estimate the linear relationship between the plasma hirudin concentration and the aPTT-to-baseline ratio, the ACT-to-baseline ratio, and the ACT in seconds.

Results

Activated Partial Thromboplastin Times and Activated Clotting Times

Fig 1 shows the aPTT over 24 hours for each group. Since the aPTT reached a plateau between 3 and 6 hours after start of the infusion, we averaged the 3-, 4-, and 6-hour measurements and related this value to the baseline aPTT to yield the plateau-to-baseline aPTT ratio. Within 30 minutes of starting the hirudin infusion, 62% to 77% of the plateau aPTT value was reached in all dosage groups. In the 0.2- and 0.3-mg·kg$^{-1}$·h$^{-1}$ groups, the aPTTs were twice baseline (60 and 64 seconds, respectively) 15 minutes after start of the infusion. The aPTT returned to baseline between 8 and 18 hours after completion of the hirudin infusion. A close dose-response relationship between hirudin dosage and plateau aPTT-to-baseline ratio was apparent across the entire dose range tested (Fig 2) and was 1.5±0.1, 2.0±0.1, 2.3±0.1, 2.7±0.1, and 2.9±0.1 for the 0.02, 0.05, 0.1, 0.2, and 0.3 mg·kg$^{-1}$·h$^{-1}$ hirudin dosage, respectively. The aPTT plateau-to-baseline ratio did not exceed 3.5 in any patient. Only 1 patient (in the 0.3-mg group) had an aPTT >100 seconds (109 seconds at 6 hours). The correlation coefficient between the aPTT-to-baseline ratio and the plasma hirudin concentrations was $r=.88$ (Fig 3A). Importantly, at a plasma hirudin concentration of 1000 ng/mL, all aPTT-to-baseline ratios were >2.0, with no overlap between baseline and treatment ratios at lower plasma hirudin concentrations.

The ACT is a bedside test of global hemostasis. Compared with the aPTT, the ACT-to-baseline ratios correlated only moderately well ($r=.44$) with the plasma hirudin concentrations (Fig 3B). The correlation coefficient of plasma hirudin concentrations versus ACTs in

![Fig 1. Plot of activated partial thromboplastin time (aPTT) during and after a 6-hour infusion of recombinant hirudin at the indicated dosages (n=6 per group) or placebo (n=9). Error bars indicate SEM.](image1)

![Fig 2. Plot of activated partial thromboplastin time (aPTT) plateau-to-baseline ratio for each of the patients studied. Plateau aPTT was mean of 3-, 4-, and 6-hour aPTTs. No change in aPTT occurred in placebo patients.](image2)
seconds was better \((r=0.80, \text{Fig 3C})\) but less sensitive than the aPTT. At a plasma hirudin level of 1000 ng/mL or slightly above, ACTs ranged from 155 to 230 seconds compared with a baseline of 75 to 180 seconds. There was considerable overlap between baseline ACT values and ACT values of plasma samples containing <1000 ng/mL of hirudin (Fig 3C).

**Prothrombin Times and Thrombin Times**

Compared with placebo (PT, 11.5), a significant \((P<0.05)\) prolongation of the PT occurred at dosage of \(\geq 0.05 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}\). PTs, in seconds, at the completion of the 6-hour hirudin infusion were 11.8±0.5 (INR=1.0), 12.3±0.7 (INR=1.1), 13.3±1.2 (INR=1.4), 14.2±0.4 (INR=1.7), and 15.8±0.9 (INR=2.3) for the 0.02, 0.05, 0.1, 0.2, and 0.3 mg \cdot kg^{-1} \cdot h^{-1} dosage, respectively. Considerable overlap in PT was seen with neighboring doses; only the 0.2 and 0.3 mg \cdot kg^{-1} \cdot h^{-1} dosages differed significantly \((P<0.05)\) in their effect on the PT. The shallow dose-response relationship compromised the ability of the PT to reflect subtle dose changes in hirudin. In contrast to the PT, the thrombin time was exquisitely sensitive to hirudin and beyond the measured range (>600 seconds) after 6 hours of hirudin in all except 2 patients from the lowest dosage group. Dose titration of hirudin, within the anticipated therapeutic ranges, was not possible with the thrombin time.

**Bleeding Time**

When compared with patients receiving placebo (bleeding time at 5.5 hours, 3.7±1.1 minutes; range, 2.3 to 5.5 minutes), bleeding times 5.5 hours into the hirudin infusion were not significantly prolonged with any of the dosages and were (in minutes) 5.3±2.3 (range, 2.5 to 9.0), 4.2±1.5 (range, 2.0 to 5.5), 4.5±1.0 (range, 3.0 to 6.0), 5.1±1.1 (range, 4.0 to 7.0), and 4.5±0.6 (range, 4.0 to 5.3) with hirudin from 0.02 to 0.3 mg \cdot kg^{-1} \cdot h^{-1}. The longest bleeding times at 5.5 hours were 9 minutes (baseline, 4 minutes), 7 minutes (baseline, 4 minutes), and 6 minutes (baseline, 4 minutes) in 3 patients receiving hirudin at 0.02, 0.2 mg, and 0.1 mg \cdot kg^{-1} \cdot h^{-1}, respectively.

**Plasma Hirudin Levels and Terminal Half-life**

Plasma hirudin levels for all five groups are shown in Fig 4. Plasma hirudin concentrations increased dose dependently to near-plateau levels. The terminal half-life for each subject and the median values for each group are shown in the Table.

**Side Effects**

No side effects were observed during or after the hirudin infusion. One patient from the 0.1 mg \cdot kg^{-1} \cdot h^{-1} group had a minor bruise after an unsuccessful venipuncture. Liver function tests (aspartate aminotransferase, alkaline phosphatase, total and direct bilirubin), serum creatinine, and urinalysis, which were all normal at baseline, were unchanged 18 hours after the infusion. No antibodies to hirudin were detected on 2-week follow-up. Platelet counts before \((237±46 \times 10^9/\text{mL})\) and 18 hours after the hirudin infusion \((240±46 \times 10^9/\text{mL})\) were not dose dependent and did not differ from those of patients on placebo.
of the anticoagulant effect of hirudin as measured by the aPTT assay. Moreover, the aPTT prolongation observed after intravenous administration of hirudin in our study closely matched the concentration-dependent aPTT prolongation reported by Markwardt et al\textsuperscript{14} and Bichler et al\textsuperscript{23} after addition of hirudin to human plasma in vitro. The ACT-to-baseline ratio was a relatively poor predictor of plasma hirudin levels at potentially therapeutic dosage for arterial thrombosis (Fig 3B), making this test insensitive for monitoring hirudin therapy. Although a better correlation was observed between plasma hirudin concentrations and ACT expressed in seconds, the data show only a small and variable increment in ACT expressed in seconds with increasing hirudin concentrations. There was also considerable overlap of treatment ACTs with baseline ACTs for plasma hirudin concentrations <1000 ng/mL. Marked overlap in all dosage groups in the dose-response curve of the PT makes the PT too insensitive for adjusting the hirudin infusion during antithrombotic therapy. The thrombin time, on the other hand, was too sensitive to hirudin and is not practical for monitoring hirudin therapy even at the lower doses anticipated to be effective and safe for the treatment of systemic venous thrombosis.

No bolus was administered prior to the 6-hour hirudin infusion, yet anticoagulation to 62% to 77% of the plateau aPTT was achieved within 30 minutes after starting the infusion. Thus, a loading bolus could be omitted in instances of lesser therapeutic urgency. Importantly, the aPTT was >100 seconds (109 seconds) in only 1 patient who received hirudin at 0.3 mg · kg\textsuperscript{-1} · h\textsuperscript{-1} and had a baseline aPTT of 35 seconds.

The reported terminal half-life (T\textsubscript{1/2b}) of hirudin after a single intravenous bolus to normal subjects (mean age, 40 years) was about 50 minutes in the study by Markwardt et al\textsuperscript{14} and 65 minutes in the study by Bichler et al.\textsuperscript{23} The terminal half-life was 2 to 3 hours in this study. These differences may be due to (1) differences in the assay of hirudin (by its anticoagulant capacity in the study by Markwardt et al, by radioimmunoassay in that by Bichler et al, and by highly specific and sensitive ELISA in our study); (2) use of plasma hirudin data for 18 hours after stopping hirudin for calculation of the terminal half-life (compared with only 3 hours in the Markwardt et al study and 7 hours in the study by

**Discussion**

This study shows that the aPTT, among common hemostatic parameters, best correlated with plasma hirudin level over a wide dosage range. The dose-response relationship between hirudin and the aPTT (Figs 1 and 2) was sensitive enough to allow close titration of hirudin to a graded intensity of anticoagulation. The narrow standard errors of the aPTT ratios at all dosages (Fig 1) further illustrates the predictability

**Terminal Half-life of Hirudin After Stopping a 6-Hour Hirudin Infusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>0.02</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.72</td>
<td>3.34</td>
<td>3.98</td>
<td>2.66</td>
<td>2.13</td>
</tr>
<tr>
<td>2</td>
<td>2.73</td>
<td>2.32</td>
<td>2.56</td>
<td>2.78</td>
<td>2.02</td>
</tr>
<tr>
<td>3</td>
<td>...</td>
<td>2.35</td>
<td>2.81</td>
<td>3.09</td>
<td>1.93</td>
</tr>
<tr>
<td>4</td>
<td>2.70</td>
<td>3.07</td>
<td>2.82</td>
<td>3.17</td>
<td>1.99</td>
</tr>
<tr>
<td>5</td>
<td>4.96</td>
<td>2.22</td>
<td>4.18</td>
<td>3.14</td>
<td>1.73</td>
</tr>
<tr>
<td>6</td>
<td>2.19</td>
<td>2.17</td>
<td>3.15</td>
<td>3.26</td>
<td>1.27</td>
</tr>
<tr>
<td>Median</td>
<td>2.72</td>
<td>2.33</td>
<td>2.98</td>
<td>3.11</td>
<td>1.96</td>
</tr>
</tbody>
</table>

Values in hours.
Bichler et al); and (3) differences in the hirudin preparations (sulfated leech hirudin, containing various isoforms, in the aforementioned studies and homogenous recombinant unsulfated hirudin in the present study). Although our study subjects had documented coronary atherosclerosis and were, on average, 20 years older than those of the studies of Markwardt et al and Bichler et al, kidney function was grossly normal (serum creatinine was 1.0±0.2 mg/dL), making decreased hirudin clearance (by renal excretion and metabolism) a less plausible explanation for the different half-lives reported.

No reports on the pharmacokinetics and anticoagulant effects of hirudin over a broad dosage range and in patients at risk for atherosclerotic complications have been available. Pathological and biochemical marker studies in these populations suggest a state of accelerated thrombin generation even at times of clinical quiescence.30-42 Thrombin generation may also vary in different thrombotic settings as suggested by different heparin dose requirements for treatment versus prevention of thrombosis and for the treatment of venous versus arterial thrombosis. Experimentally, hirudin dose requirements also vary with different pathogeneses of thrombosis and may indicate different amounts of, or access to, thrombin generated.43 These differences in thrombin turnover may be of limited importance to the pharmacokinetics and anticoagulant effects of hirudin at dosage anticipated for arterial thrombus. However, they may have implications for the use of low-dose hirudin for treatment of venous thrombosis and for preventing intravascular thrombin, which was explored in the present patient group in another context.44 Thus, we studied patients with documented coronary artery disease and administered hirudin over a 15-fold dosage range (0.02 to 0.3 mg · kg⁻¹ · h⁻¹).

At anticoagulant dosages effective in a variety of animal thrombosis models,10,13,25-27 no side effects occurred during or after the hirudin infusion. Although the clinical implication of prolonged bleeding times is still debated,45,46 the finding of no prolonged bleeding times from baseline is reassuring. Preservation of the bleeding time, at anticipated antithrombotic hirudin dosage, could be due to two factors. First, doses of hirudin that prolonged the aPTT to two to three times control in pigs prevented intraluminal thrombus10 but did not prevent thrombus formation within the fissures and tears of the deeply injured arterial wall (Reference 10 and unpublished observations from our laboratory). The residual intramural thrombus could be protective against bleeding. Second, although thrombin-induced platelet activation is critical for thrombosis after deep arterial injury,10,23 local vasoconstriction and connective tissue components (including collagen) may be more important contributors to the initial platelet plug in microvascular and perivascular locations.45 Moreover, species differences in the hemostatic system and location of the skin incision make interspecies comparisons difficult. Thus, heparin and hirudin at all antithrombotic and subantithrombotic doses tested significantly but mildly prolonged the bleeding time after an ear incision in the pig (without causing excess bleeding during transfemoral carotid angioplasty).10,47 but hirudin did not prolong the bleeding time in humans in our study. Hirudin, at plasma level of about 1000 ng/mL, substani-

tially reduced platelet deposition on endarterectomized aorta segments in a baboon arteriovenous shunt model and prevented occlusion of the shunts in all animals, whereas heparin at 1000 U/kg IV bolus did not.24 Of note, the aPTT in this model was 10-fold control after 100 U/kg heparin, while the bleeding time was minimally prolonged after an IV heparin bolus of 1000 U/kg. The bleeding time was not prolonged at an effective antithrombotic hirudin plasma concentration of 1000 ng/mL (aPTT twice control), although it was prolonged to 12.1 and 13.8 minutes with plasma hirudin concentrations of 5.4 and 22.6 pg/mL.24 The clinical benefit and risk of hirudin dosage in excess of 0.3 mg · kg⁻¹ · h⁻¹ cannot be extrapolated from these animal studies. Furthermore, pilot studies suggest a significant therapeutic advantage of hirudin at 0.2 mg · kg⁻¹ · h⁻¹ over heparin in patients receiving recombinant tissue-type plasminogen activator and aspirin for acute myocardial infarction20 and at 0.2 to 0.3 mg · kg⁻¹ · h⁻¹ for patients with unstable angina and angiographic thrombus (E. Topol, V. Fuster, J.H. Chesebro, unpublished data). Note that these dosages match the aPTTs of 2.5 to 3 times control (75 to 90 seconds) effective for thrombus after deep arterial injury in pigs and in baboon.10,24

The "antithrombotic/anticoagulant (aPTT) profile"27 of antithrombins appears to be related to the ability of antithrombins to inhibit clot-bound thrombin (where the antithrombin III–binding sites on thrombin are masked)48-50 and depends on the affinity for thrombin and the interaction of the inhibitor with both the catalytic site and the anion-binding exosite of thrombin.49,51 Slight decreases in affinity of hirudin for thrombin, through point mutations in amino acid position 47, diminished its inhibitory effect on platelet aggregation while leaving its potency for inhibiting plasma clotting unchanged.52 Although the antithrombotic/anticoagulant profile also varies with the type of thrombus under examination (making comparison of different antithrombins in different animal models difficult), the profile is high with hirudin (and its analogue hirulog) and low with heparin.10,23,48,51 Synthetic thrombin inhibitors that bind to thrombin less tightly than hirudin (with dissociation constants several orders of magnitude larger than hirudin)53 have not completely prevented arterial thrombus or reocclusion after lysis, even when anticoagulant doses that increased the aPTT to more than five times control were used, as in two recent studies with argatroban.11,54 Because arterial thrombosis in humans is platelet rich and usually occurs in vascular regions rich in thrombin-generating tissue factor,55 human dose-ranging studies should probably match aPTTs necessary for reducing or preventing arterial thrombus formation in large animals after deep (or plaque) injury. This is not always done as in the case of argatroban, which was recently tested in six healthy men who received a dose of argatroban yielding an aPTT of 1.6 times baseline,56 which is lower than the anticipated therapeutic range. In contrast, hirudin at the aPTT-prolonging doses in this study were effective antithrombotics in animals and in pilot studies in humans. Assessment of the antithrombotic activity and safety profile of ascending hirudin doses with direct measurement of arterial thrombus (such as by ¹¹¹In-platelet scintigraphy57,58) in small numbers of patients may allow a single objectively chosen dosage to be tested in future clinical
efficacy trials. The optimal duration of hirudin therapy and the mechanism of reactivation of coagulation following antithrombin therapy, including the possibility of a rebound phenomenon (reported following an infusion of argatroban to patients with unstable angina), are currently being investigated. Through its additional potential as a probe for intravascular thrombin, hirudin could contribute to a better understanding of prothrombin activation, both during and early after administration of the antithrombin.44

In summary, a 6-hour IV infusion of hirudin resulted in a dose-dependent, sensitive prolongation of the aPTT that correlated closely with blood levels of hirudin and reached a plateau 3 to 4 hours after starting the infusion. The thrombin time was overly sensitive for monitoring the dosage of hirudin. Although PT and ACT were too insensitive for routine, dose titration, the PT distinguished patients with infusions of 0.2 and 0.3 mg · kg⁻¹ · h⁻¹ and thus may better identify higher blood levels of hirudin than the aPTT, which plateaus at levels >2000 ng/mL. Thus, among the readily available hemo-

din-analogue effect of hirudin at anticipated therapeutic doses. The terminal half-life of hirudin in these patients is 2 to 3 hours. At anticoagulant dosages highly effective in animal models of thrombus prevention and lysis (aPTT two to three times control), a 6-hour infusion of hirudin was well tolerated in our predominantly male group of patients with established atherosclerosis.

Acknowledgments

 Funded by a grant from CIBA-GEIGY, Summit, NJ. The authors thank Gilbert Lefèvre, Laboratoires CIBA-GEIGY, Centre de Recherche Biopharmaceutique, Rueil-Malmaison, France, for measuring plasma hirudin levels; Johann Bichler for performing the IgE and IgG tests; and Cindy Murphy for excellent secretarial skills.

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Circulation. 1993;88:2015-2022
doi: 10.1161/01.CIR.88.5.2015

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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