Abstract—In patients with unstable angina, intravenous heparin reduces thrombin activity but does not influence thrombin generation. Recombinant hirudin, a direct thrombin inhibitor, may be more effective in inhibiting both thrombin generation and activity. We measured the plasma levels of prothrombin fragment 1+2 (a marker of thrombin generation) and fibrinopeptide A (a marker of thrombin activity) in 67 patients with unstable angina enrolled in the GUSTO (Global Use of Strategies to Open Occluded Coronary Arteries) IIb trial who were receiving either recombinant hirudin (31 patients) or heparin (36 patients). Blood samples were obtained at baseline (before any treatment), after 3 to 5 days of study drug infusion (immediately before discontinuation), and 1 month later. In the patients receiving recombinant hirudin, the prothrombin fragment 1+2 levels measured immediately before drug discontinuation were significantly lower than at baseline ($P=0.0014$), whereas they had not changed in the patients receiving heparin; at this time point, the difference between patients receiving hirudin and those receiving heparin was statistically significant ($P=0.032$). One month later, the prothrombin fragment 1+2 levels in both groups were similarly persistently high and did not differ from baseline. Fibrinopeptide A plasma levels at the end of infusion were significantly lower than at baseline in both treatment groups ($P=0.005$ for hirudin and $P=0.042$ for heparin) and remained lower after 1 month ($P=0.0001$ for both hirudin and heparin). The fibrinopeptide A plasma levels were not different between patients treated with hirudin versus heparin at baseline, at the end of infusion, and after 1 month. Thus, in patients with unstable angina, in vivo thrombin generation and activity are reduced during intravenous infusion of recombinant hirudin. However, the inhibition of thrombin generation is not sustained, and after 1 month, the majority of patients have biochemical signs of increased thrombin generation. (Arterioscler Thromb Vasc Biol. 2000;20:2162-2166.)

Key Words: hirudin ■ thrombin generation ■ unstable angina

Intravenous heparin has become a recommended therapy for patients with unstable angina because it leads to an improved clinical outcome.¹ Heparin’s binding to antithrombin produces a conformational change in antithrombin that considerably accelerates the ability of the latter to inactivate coagulation enzymes.²,³ This is particularly true in the case of thrombin, which plays a critical role in the amplification of the coagulation cascade by activating factor V, factor VIII,⁴–⁶ and platelets.⁷,⁸ However, although the levels of fibrinopeptide A (a sensitive marker of thrombin activity) decrease in heparin-treated patients with unstable angina,⁹–¹¹ the plasma levels of prothrombin fragment 1+2 (a sensitive marker of thrombin generation) are not reduced.¹² Because the self-amplification of thrombin through the above-mentioned activation of platelets and factors V, VIII, and X is thought to be critical for the generation of critical concentrations necessary for effective hemostasis and thrombosis,⁵,¹³ increased thrombin generation may partially contribute to the persistent thrombotic risk during and after heparin treatment.¹⁴

Recombinant hirudin is a 65–amino acid thrombin inhibitor that forms an irreversible complex by directly binding to thrombin. Unlike heparin, recombinant hirudin does not need antithrombin as a cofactor, and hirudin also inhibits clot-bound thrombin. Such blocking of all thrombin activity should lead to the interruption of thrombin self-generation by inhibiting the positive feedback of the multiple mechanisms involved. The aim of this study was to compare the effects of heparin and recombinant hirudin on in vivo thrombin gener-
ation and activity during drug infusion and after 1 month in a cohort of patients with unstable angina enrolled in the GUSTO IIb trial.

Methods

Study Population
The study population consisted of patients with unstable angina or non–Q-wave myocardial infarction enrolled in the GUSTO IIb trial at the Division of Cardiology, Ca’ Granda Niguarda Hospital (Milan), the Division of Cardiology IRCCS Policlinico San Matteo (Pavia), Ospedale GB Morgagni (Forlì), and Ospedale Civile (Ravenna), Italy. All of the patients had to have reported symptoms of cardiac ischemia at rest within 12 hours of their admission and to have shown electrocardiographic signs of acute myocardial ischemia at presentation: ie, transient ST-segment elevation or depression >0.5 mm or a persistent, definite T-wave inversion >1 mm. Patients were excluded from the GUSTO IIb study if they were taking warfarin at the time of enrollment or if they had active bleeding, a history of stroke, a contraindication to heparin therapy, renal insufficiency, or systolic blood pressure >110 mm Hg. Of the eligible patients, those receiving heparin or thrombolytic therapy at the time of enrollment or who had a severely limited venous access were excluded from the hemostatic evaluation.

Study Protocol
Each patient enrolled in the GUSTO IIb study received either intravenous heparin or desulfato recombinant hirudin (Desirudin, Ciba-Geigy) for a minimum of 3 and a maximum of 5 days according to the study protocol, with the dose being adjusted to maintain an activated partial thromboplastin time between 60 and 85 seconds; all of the patients received aspirin (165 to 325 mg) before the start of the study drug. Any associated treatment was given at the discretion of the patients’ individual physicians and was not dictated by the study protocol. A baseline blood sample was obtained before any treatment was started, including the study drug, and another sample was obtained after 3 to 5 days, immediately before study drug discontinuation. At a follow-up visit after 1 month, a further blood sample was obtained. Long-term treatment was left to the discretion of the patients’ individual physicians and in all patients included aspirin and excluded oral anticoagulants. The study was approved by the Institutional Review Board of the Ca’ Granda Niguarda Hospital (Milan, Italy), and written informed consent was obtained from all of the subjects. All of the clinical studies and informed consent procedures were also approved by the Committee on Clinical Investigations of the Beth Israel Hospital (Boston, Mass).

Blood Sampling and Handling
Clean venipunctures were performed by specially trained investigators using 19-gauge butterfly infusion sets and a 2-syringe technique. Inadequate blood samples were prospectively excluded. After the first 4 mL of blood was discarded, the samples were placed directly into refrigerated Vacutainers containing an anticoagulant mixture consisting of a thrombin inhibitor (Phe-Pro-Arg chloromethyl ketone), EDTA, and aprotonin (Byk-Sangtec); the ratio of anticoagulant to blood was 1:9, vol/vol. The samples were immediately centrifuged at 2500g, and the plasma was divided into aliquots, snap-frozen, and stored at −80°C until analyzed.

Biochemical Determinations
All of the samples were centrally analyzed by investigators who were unaware of the clinical data. The plasma levels of prothrombin fragment 1+2 were measured by using a double-antibody radioimmunoassay as previously described16; this method has an interassay coefficient of variation of ~8%. Plasma fibrinopeptide A concentrations were determined in duplicate by means of an enzyme immunoassay in plasma extracted twice with bentonite to remove fibrinogen (Diagnosticia Stago); this technique has an interassay coefficient of variation of ~5%. Because fibrinopeptide A is known to be susceptible to in vitro sampling artifacts, blood samples with fibrinopeptide A levels >30 nmol/L were not used for the determination of either marker because it was surmised that they were affected by poor sample acquisition, handling, or storage.17

Statistical Analysis
Because the plasma levels of the coagulation system markers are not normally distributed, repeated measures were compared by means of Friedman’s test, and subsequent pairwise comparisons with baseline were made with Wilcoxon’s signed-rank test. The Mann-Whitney U test was used to test the difference between groups. The descriptive statistics include mean values and standard deviations, or median values and 25th and 75th percentiles. The number of patients who exhibited plasma concentrations of prothrombin fragment 1+2 and fibrinopeptide A above the upper normal limits was calculated by determining the 95th percentile of the distribution in the control group of healthy individuals, which was set at 1.02 nmol/L for prothrombin fragment 1+2 and 2.2 nmol/L for fibrinopeptide A.18 Prevalences were compared by means of the χ² test. All of the tests were 2-tailed, and a probability value <0.05 were regarded as statistically significant.

Results

Patient Characteristics
Biochemical evaluation was attempted in 78 consecutive patients, 42 of whom received heparin and 36, recombinant hirudin. Eleven patients did not undergo complete evaluation for the following reasons: death within 1 month (4 patients), inadequate blood sampling (6 patients), and severely limited venous access (1 patient). A complete set of 3 samples (baseline, immediately before study drug discontinuation, and after 1 month) was therefore available for 67 patients, 31 who were treated with recombinant hirudin and 36 with heparin. Their demographic and clinical characteristics are shown in Table 1. The mean duration of recombinant hirudin and heparin therapy was respectively, 82±11 and 77±9 hours (P=NS). The mean activated partial thromboplastin time at baseline was 23±5 seconds for patients receiving hirudin and 24±7 seconds for those receiving heparin (P=NS), whereas the activated partial thromboplastin time immediately before drug discontinuation was 74±22 seconds and 69±27 seconds, respectively (P=NS).

In Vivo Thrombin Generation and Activity in Patients Receiving Recombinant Hirudin
The median and 25th and 75th percentiles of the plasma prothrombin fragment 1+2 and fibrinopeptide A levels at

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics</th>
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<tr>
<td></td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Males, n (%)</td>
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<tr>
<td>Height, cm</td>
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<td>Weight, kg</td>
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<tr>
<td>Positive family history, n (%)</td>
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<td>Smokers, n (%)</td>
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<td>Hypertension, n (%)</td>
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<td>Hyperlipidemia, n (%)</td>
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<td>T-wave inversion</td>
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<tr>
<td>ST-segment depression</td>
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<td>ST-segment elevation</td>
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Values are either mean±SD or n (%).
TABLE 2. Median Plasma Values (25th to 75th Percentiles) of Prothrombin Fragment 1+2 and Fibrinopeptide A at Baseline, Before Drug Discontinuation, and After 1 Month in Patients Receiving Either Heparin or Hirudin

<table>
<thead>
<tr>
<th>Biochemical Marker/Treatment</th>
<th>Baseline</th>
<th>Before Drug Discontinuation</th>
<th>After 1 Month</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin fragment 1+2, nmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirudin (n=31)</td>
<td>1.20 (0.94–1.47)</td>
<td>0.95 (0.76–1.15)**</td>
<td>1.34 (0.98–1.15)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Heparin (n=36)</td>
<td>1.07 (0.84–1.36)</td>
<td>1.16 (0.91–1.39)</td>
<td>1.26 (0.95–1.74)</td>
<td>0.024</td>
</tr>
<tr>
<td>Fibrinopeptide A, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirudin (n=31)</td>
<td>2.3 (1.2–2.6)</td>
<td>0.9 (0.7–1.1)**</td>
<td>1.0 (0.6–1.2)#</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heparin (n=36)</td>
<td>2.3 (1.15–4.2)</td>
<td>1.02 (0.8–1.8)**</td>
<td>1.05 (0.55–1.3)d</td>
<td>0.0048</td>
</tr>
</tbody>
</table>

*P refers to comparison within the treatment group.  †P refers to comparison at each time point between the 2 treatment groups.  ‡P=0.0014 vs baseline.  §P=0.0001 vs before drug discontinuation.  ¶P=0.040 vs baseline.  ††P=0.0005 vs baseline.  #P=0.0001 vs baseline.  **P=0.042 vs baseline.

different time points for patients treated with recombinant hirudin are given in Table 2. There was a significant decrease of plasma prothrombin fragment 1+2 during recombinant hirudin infusion (P=0.0014). After 1 month, the levels were similar to those found at baseline (P=NS) but were significantly higher than those found before drug discontinuation (P=0.0001). The prevalence of abnormal levels immediately before drug discontinuation was significantly lower than at baseline: 22 patients (71%) versus 14 (45%, P=0.039); however, after 1 month, the prevalence of abnormal values (21 patients, 70%) was similar to that observed at baseline.

Median fibrinopeptide A levels decreased significantly after a 72-hour infusion of recombinant hirudin (P=0.0005) and were still significantly lower than baseline after 1 month (P=0.0001). There was also a significant decrease in the prevalence of abnormal levels at the same time points: 17 patients (57%) at baseline, 4 (13%) immediately before drug discontinuation, and 1 (3%) after 1 month (P=0.0001).

In Vivo Thrombin Generation and Activity in Patients Receiving Heparin

The median and 25th and 75th percentile values of the plasma prothrombin fragment 1+2 and fibrinopeptide A levels at different time points for the patients treated with heparin are given in Table 2. There was no difference between plasma levels of prothrombin fragment 1+2 observed at baseline and those found before heparin discontinuation. After 1 month, plasma prothrombin fragment 1+2 levels were significantly increased compared with baseline (P=0.040). There was no difference in the prevalence of abnormal prothrombin fragment 1+2 levels at the different time points: 18 patients (50%) had abnormal plasma prothrombin fragment 1+2 levels at baseline, 21 (60%) at the time of drug discontinuation, and 26 (72%) after 1 month.

In comparison with baseline, there was a significant decrease of plasma fibrinopeptide A levels at the end of the infusion (P=0.042) and after 1 month (P=0.0001). At the same time points, there was also a significant decrease in the prevalence of abnormal plasma fibrinopeptide A levels, which were found in 19 patients (54%) at baseline, 6 (17%) immediately before drug discontinuation, and 3 (9%) after 1 month (P=0.0001).

Comparison of Heparin and Recombinant Hirudin With Respect to Their Effects on In Vivo Thrombin Generation and Activity

The baseline plasma prothrombin fragment 1+2 and fibrinopeptide A levels were similar in the 2 treatment groups. The plasma prothrombin fragment 1+2 levels decreased during treatment in the patients receiving recombinant hirudin but did not change in those receiving heparin; thus, the postinfusion median plasma prothrombin fragment 1+2 levels were significantly lower in the former (P=0.032). However, after 1 month, the levels of plasma prothrombin fragment 1+2 had increased to similar levels in both groups and were not different from baseline. Plasma fibrinopeptide A decreased to within normal limits in both groups and remained low after 1 month, without any difference between heparin- and hirudin-treated patients.

Discussion

The antithrombotic action of heparin is dependent on antithrombin; in vivo studies have shown that the plasma levels of fibrinopeptide A (a marker of thrombin activity) dramatically decrease after heparin treatment.8–10 However, heparin cannot inactivate clot-bound thrombin, which retains its catalytic activity against fibrinogen, factor V, and factor VIII and activates the expression of cell binding sites on platelets for the assembly of the tenase and prothrombinase vitamin K–dependent complexes on the platelet membranes.4–8,10–14 Furthermore, heparin is inactivated by platelet factor 4.25 All of these reasons may account for the fact that thrombin generation in vivo does not change during heparin treatment,12 and this high thrombin generation may underlie the persistent thrombosis risk observed after heparin discontinuation.14
Recombinant hirudin interacts directly with thrombin, without depending on antithrombin, and inactivates both thrombus-bound and soluble thrombin in vitro; thus, it should be more effective in vivo in blocking thrombin generation through the obstruction of its feedback-amplifying mechanisms. In experimental animal models, recombinant hirudin has been shown to be much more effective than high-dose heparin and aspirin in reducing platelet deposition and thrombosis after catheter-induced vascular injury, but it is not known whether direct thrombin inhibition leads to a decrease in thrombin generation in a clinical setting.

This study shows that in patients with unstable angina, a 3- to 5-day infusion of recombinant hirudin leads to a decrease in thrombin generation that is not observed in the patients receiving heparin. In patients with acute coronary syndromes, higher plasma prothrombin fragment 1+2 levels are associated with an increased risk of in-hospital events; thus, it can be surmised that the early reduction in cardiac events observed in patients receiving recombinant hirudin may be related to its ability to decrease thrombin generation. This finding is different from the results of other studies, which revealed no effect of recombinant hirudin on thrombin generation despite decreased thrombin activity. This discrepancy could be due to the longer treatment used in our study or to the fact that our study population showed signs of increased thrombin generation at baseline, thereby indicating that coronary thrombosis was likely to be the main pathogenetic mechanism. It is interesting to note that in a recent pooled analysis of all large trials of hirudin versus heparin in acute coronary syndromes, the greater risk reduction (28% at 72 hours) was observed in patients not receiving thrombolytic therapy, which is similar to the population represented in the present study.

One month after treatment, thrombin generation returned high levels in both heparin- and hirudin-treated patients. It is tempting to speculate that the attenuation of the short-term favorable clinical effect of recombinant hirudin over heparin observed in the GUSTO IIb and OASIS-2 (Organization to Assess Strategies for Ischemic Syndrome-2) trial, with the occurrence of a significant number of additional ischemic events after drug discontinuation, may be due to its inability to persistently inhibit thrombin generation. Recent data have shown that the healing process of culprit lesions in acute coronary syndromes takes a long time, and angiographic signs of persistent plaque instability and thrombosis can be observed even in the absence of symptoms. These findings are in keeping with the result of previous studies showing that the hemostatic mechanism, including platelets, remains activated up to 6 months after an episode of unstable angina or myocardial infarction.

Continuous thrombin generation may be due to the unhealed wall injury with exposure of tissue factor, the chronic activity of the underlying disease, the persistent platelet activation, and their interplay, which suggest that prolonged inhibition of the hemostatic mechanism may be needed to include the period of healing of the culprit lesion and to allow the coagulation system to return to baseline levels of responsiveness, thereby reducing the risk of recurrent ischemic events.

References


In Vivo Thrombin Generation and Activity During and After Intravenous Infusion of Heparin or Recombinant Hirudin in Patients With Unstable Angina Pectoris
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