Activation of the Hemostatic Mechanism During Thrombolysis in Patients With Unstable Angina Pectoris

By Piera Angelica Merlini, Diego Ardissino, Kenneth A. Bauer, Luigi Oltrona, Alessandra Spinola, Paolo Diotallevi, Robert D. Rosenberg, and Pier Mannuccio Mannucci

In patients with myocardial infarction, thrombolytic therapy induces a paradoxical activation of the hemostatic mechanism. In patients with unstable angina, the effect of thrombolysis on the coagulation cascade is unknown. We prospectively measured the plasma concentrations of prothrombin fragment 1 + 2 and fibrinopeptide A in consecutive patients with unstable angina randomized to receive placebo alone (n = 23), streptokinase 1,500,000 IU over 1 hour followed by a 48-hour placebo infusion (n = 21), or streptokinase 250,000 IU over 1 hour followed by a continuous infusion of 100,000 IU per hour over 48 hours (n = 20). All the patients received intravenous heparin for 72 hours. The plasma levels of the different markers were measured at baseline, 90 minutes, 24 hours, and 48 hours after the start of therapy. The median baseline plasma concentrations of prothrombin fragment 1 + 2 and fibrinopeptide A were similar in the three treatment groups. In comparison with placebo, an increase in plasma prothrombin fragment 1 + 2 and fibrinopeptide A, was observed after 90 minutes in the two groups receiving thrombolysis. After 24 and 48 hours, the prothrombin fragment 1 + 2 levels remained significantly higher only in the patients receiving the 48-hour streptokinase infusion. In patients with unstable angina, thrombolytic therapy induces an activation of the hemostatic mechanism, despite concomitant heparin administration; in those receiving a prolonged streptokinase infusion, the activation of coagulation persists for as long as the drug is administered.

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Exclusion Criteria

Patients with comorbid conditions known to alter coagulation system activity or to decrease the clearance of activation fragments, as well as those who were taking drugs affecting the function of the hemostatic mechanism, were deemed ineligible for the study. Of the 100 patients included in the original clinical trial, those with the following conditions were excluded from the present study: concomitant peripheral vascular disorders or valvular heart disease (6 patients), start of anticoagulant therapy before baseline blood sampling (14 patients), severely limited venous access (16 patients).

Study Protocol

Screening for the study was done in the emergency room. After the patients were included in the study, venous blood samples were collected for baseline biochemical and coagulation analyses. The enrolled patients were randomly allocated to receive one of the following treatments: (1) an infusion of placebo over 1 hour, followed by a further infusion of placebo for another 48 hours (the placebo group); (2) an infusion of streptokinase 1,500,000 IU over 1 hour, followed by a placebo infusion for 48 hours (the acute

From the 2nd Division of Cardiology, Ca’ Granda Niguarda Hospital, Milan, Italy; the Division of Cardiology, I.R.C.C.S Policlinico San Matteo, Pavia, Italy; Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, MA; the Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, and the Institute of Internal Medicine, I.R.C.C.S Maggiore Hospital, University of Milan, Italy; and the Department of Biology, Massachusetts Institute of Technology, Cambridge.

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Address reprint requests to Piera Angelica Merlini, MD, 2nd Division of Cardiology, Ospedale Ca’ Granda Niguarda, 20162 Milano, Italy.

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streptokinase group); or (3) an infusion of 250,000 U over 1 hour followed by a prolonged infusion of 100,000 U per hour for the following 48 hours (the prolonged streptokinase group). All the patients received a concomitant intravenous heparin bolus of 5,000 U before the start of the assigned treatment, followed by a 72-hour infusion of 1,000 U per hour to maintain an activated partial thromboplastin time of more than twice the control values. The associated treatments were a combination of intravenous nitroglycerin (0.1 to 1 μg/kg/min) and/or diltiazem (1 to 6 μg/kg/min) or oral beta-blockers (atenolol) 50 to 100 mg per day). None of the patients received aspirin during the first 48 hours. The patients were followed up for the occurrence of adverse outcome events for the first 72 hours after the start of the assigned treatment. Twelve-lead electrocardiograms were recorded whenever chest pain occurred. Creatine kinase levels were measured every morning, and every 4 hours after any episode of chest pain. Continuous electrocardiographic Holter monitoring for 72 hours was started immediately after randomization, simultaneously with the start of the assigned therapy. Additional blood samples for coagulation activation markers were obtained at 90 minutes, 24 hours, and 48 hours.

**Blood Sampling and Handling**

Clean venipunctures were performed by three specially trained investigators using 19-gauge butterfly infusion sets and a two-syringe technique. Inadequate blood samples were prospectively excluded. After the first 4 mL of blood were discarded, the samples were placed directly into refrigerated vacucontainers containing an anticoagulant composed of a thrombin inhibitor, EDTA, and apotinin (Byk-Sangtec, Diezenbach, Germany). The ratio of anticoagulant to blood was 1:9 (v/vol). The blood samples were immediately centrifuged at 2,500g for 25 minutes at 4°C; the platelet-free plasmas were frozen on dry ice and stored at -80°C until analyzed.

**Biochemical Determinations**

All the samples were analyzed by investigators who were unaware of the clinical data. The plasma levels of prothrombin fragment 1 + 2 were measured using a double-antibody radioimmunoassay as previously described. This method has an interassay coefficient of variation of about 8%. Plasma fibrinopeptide A concentrations were determined in duplicate by means of an enzyme immunoassay in plasma extracted twice with bentonite to remove the fibrinogen (Diagnostica Stago, Asnieres, France). This technique has an interassay coefficient of variation of about 5%.

**Adverse Outcome Events**

Adverse outcome events were considered cardiac death, Q wave or non-Q wave myocardial infarction, or persistent ischemia at Holter monitoring. Cardiac death was defined as death due to cardiac causes. Q wave myocardial infarction was defined as a prolonged episode of chest pain, accompanied by a subsequent increase in creatine kinase levels to more than twice the upper normal limit with a corresponding increase in the MB fraction and the development of Q waves on the standard 12-lead electrocardiogram. The diagnosis of non-Q wave myocardial infarction required only the first two characteristics. Persistent ischemia was considered to have occurred in the event of at least one symptomatic or asymptomatic ischemic attack during the 72-hour Holter monitoring period.

**Holter Monitoring**

Holter monitoring was performed with a Delmar Avionics Electrocadiooder model 445 with a frequency response of 0.05 to 100 Hz, which meets the specifications of the American Heart Association. The leads showing the most obvious electrocardiographic changes during the spontaneous attacks were monitored. Leads with abnormal waves or significant ST segment shifts were avoided. The system was calibrated before and after each placement. The tapes were analyzed at 60 times real time under continuous visual inspection and an episode of transient ischemia was defined as 1 mm or more of ST segment elevation or depression occurring 80 milliseconds after the J point, lasting for at least 1 minute and separated from other episodes by at least 1 minute. When a significant ST segment change was noted on the monitor, the episode was recorded on electrocardiographic paper at 25 mm/s.

**Coronary Arteriography**

Selective coronary arteriography was performed in multiple views by the Sones or Judkins technique, after premedication with 10 mg of diazepam. A narrowing in diameter in the coronary arteries of more than 50% was considered as representing significant coronary stenosis. The patients were classified as having 1, 2, or 3-vessel disease according to the number of vessels showing significant coronary stenoses.

**Informed Consent**

The study was approved by the Institutional Review Board of the Ca' Granda Niguarda Hospital (Milan, Italy) and informed consent was obtained from all subjects. All the clinical studies and informed consent procedures were also approved by the Committee on Clinical Investigations of the Beth Israel Hospital (Boston, MA).

**Statistical Analysis**

The deviations of the plasma concentrations of prothrombin fragment 1 + 2 and fibrinopeptide A from a normal distribution were tested by calculating the coefficients of skewness and kurtosis. Given that the plasma levels of the coagulation system markers were found to be non-normally distributed, the Kruskal-Wallis one-way analysis of variance was used to test between-group differences; subsequent pairwise comparisons were made using the Mann-Whitney U test with a downward adjustment of the alpha level to compensate for multiple comparisons. Repeated measures were compared by means of the Friedman test and subsequent pairwise comparisons with baseline were made using the Wilcoxon signed-rank test with a downward adjustment of the alpha level to compensate for multiple comparisons.

Descriptive statistics include means and standard deviations, or medians and interquartile ranges, as appropriate. All the tests presented are two-tailed and P values of below <.05 have been regarded as statistically significant.

**RESULTS**

We investigated 64 patients with Braunwald's Class IIIIB unstable angina pectoris: 23 in the placebo group, 21 in the acute streptokinase group, and 20 in the prolonged streptokinase group. No differences were detected in the clinical, electrocardiographic, or angiographic characteristics of the three treatment groups (Table 1).

**Coagulation Activation Markers During Thrombolysis**

**Prothrombin fragment 1 + 2.** The median values and interquartile ranges of the prothrombin fragment 1 + 2 plasma levels of the three groups at the various time points are presented in Table 2. At baseline, there were no differences in the median prothrombin fragment 1 + 2 levels of the three groups. After 90 minutes, significantly higher
Clinical characteristics

Table 1. Clinical and Angiographic Characteristics of the Patients

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Placebo (N = 23)</th>
<th>Acute Streptokinase (N = 21)</th>
<th>Prolonged Streptokinase (N = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>60 ± 8</td>
<td>59 ± 7</td>
<td>58 ± 8</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>21 (81%)</td>
<td>17 (80%)</td>
<td>18 (90%)</td>
</tr>
<tr>
<td>Smokers</td>
<td>19 (82%)</td>
<td>17 (81%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (43%)</td>
<td>10 (47%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Diabetes melitus</td>
<td>2 (8%)</td>
<td>1 (7%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>209 ± 36</td>
<td>225 ± 40</td>
<td>222 ± 34</td>
</tr>
<tr>
<td>Prior MI</td>
<td>4 (17%)</td>
<td>9 (42%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Prior angina</td>
<td>13 (51%)</td>
<td>9 (42%)</td>
<td>5 (35%)</td>
</tr>
</tbody>
</table>

Electrocardiographic characteristics

Table 2. Plasma Concentrations of Prothrombin Fragment 1 + 2 and Fibrinopeptide A in Patients Receiving Heparin or Acute Streptokinase or Prolonged Streptokinase

<table>
<thead>
<tr>
<th>Prothrombin Fragment 1 + 2 [nmol/L]</th>
<th>Heparin (23 pts)</th>
<th>Streptokinase 1 h (21 pts)</th>
<th>Streptokinase 48 h (20 pts)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.2</td>
<td>1.13</td>
<td>1.12</td>
<td>NS</td>
</tr>
<tr>
<td>90 minutes</td>
<td>(0.89-1.53)</td>
<td>(0.88-1.49)</td>
<td>(0.89-1.32)</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>1.13</td>
<td>2.57</td>
<td>2.77</td>
<td>0.0001</td>
</tr>
<tr>
<td>48 hours</td>
<td>(0.89-1.48)</td>
<td>(1.92-3.17)</td>
<td>(2.24-3.13)</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>NS</td>
<td>0.93-1.94</td>
<td>(3.75-2.57)</td>
<td></td>
</tr>
<tr>
<td>Fibrinopeptide A (nmol/L)</td>
<td>Heparin (23 pts)</td>
<td>Streptokinase 1 h (21 pts)</td>
<td>Streptokinase 48 h (20 pts)</td>
<td>P*</td>
</tr>
<tr>
<td>Baseline</td>
<td>2.3</td>
<td>3.0</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>90 minutes</td>
<td>(1.2-4.3)</td>
<td>(1.8-4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>1.4</td>
<td>3.9</td>
<td>2.7</td>
<td>0.0031</td>
</tr>
<tr>
<td>48 hours</td>
<td>(0.8-2.3)</td>
<td>(1.7-4.9)</td>
<td>(1.3-2.6)</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>1.8</td>
<td>1.6</td>
<td>2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinopeptide A (nmol/L)</td>
<td>Heparin (23 pts)</td>
<td>Streptokinase 1 h (21 pts)</td>
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<tr>
<td>Baseline</td>
<td>1.4</td>
<td>3.9</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>90 minutes</td>
<td>(1.0-3.4)</td>
<td>(1.7-3.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td>1.8</td>
<td>1.6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>48 hours</td>
<td>(1.3-2.5)</td>
<td>(1.2-3.3)</td>
<td>(1.3-4.3)</td>
<td></td>
</tr>
</tbody>
</table>

Values are medians with the interquartile range in parentheses.

* Values refer to comparisons between the three treatment groups at each time point.

† Values refer to comparisons within each group at different time points.
Coagulation Activation Markers and Adverse Outcome Events

During the 72 hours of the study period, 3 patients developed Q-wave myocardial infarction: 1 in the acute streptokinase group and 2 in the prolonged streptokinase group. Six patients in the placebo group had persistent ischemia during the first 24 hours, 5 between the 24th and 48th hour, and 6 between the 48th and the 72nd hour. In the acute streptokinase group 7 patients had persistent ischemia during the first 24 hours, 1 had asymptomatic ischemia between the 24th and 48th hour, and none had persistent ischemia between the 48th and the 72nd hour. In the prolonged streptokinase group, 8 patients developed persistent ischemia during the first 24 hours, 2 between the 24th and 48th hour, and 2 between the 48th and 72nd hour. The distribution of adverse outcome events (myocardial infarction, symptomatic or asymptomatic persistent myocardial ischemia) over the 72-hour study period in the three treatment groups is shown in Fig 1. Clustering of events is observed during the first 24 hours in the acute streptokinase group and in the first 48 hours in the prolonged streptokinase group, whereas there was an even distribution of events throughout the 72 hours of the study period in the placebo group. The median fibrinopeptide A levels measured in the plasma sample drawn before the occurrence of an adverse outcome event were higher (2.6 nmol/L; interquartile range 1.5 to 4.3) than the median fibrinopeptide A levels measured in the plasma sample drawn at the corresponding time point in the patients who did not develop an event (1.8 nmol/L; interquartile range 1.1 to 3.8, \( P = .003 \)). No difference was found between the levels of prothrombin fragment 1 + 2 measured in the plasma sample drawn before the occurrence of an event (1.57 nmol/L; interquartile range 1.11 to 2.71) and those measured in the plasma drawn at the corresponding time point in the patients who did not develop events (1.53; interquartile range 0.9 to 2.1; \( P = NS \)).

DISCUSSION

This study shows that the administration of streptokinase and concomitant intravenous heparin to patients with acute unstable angina induces an activation of the hemostatic mechanism. In comparison with baseline, there was no change in thrombin generation in the placebo group, but there was an early increase in thrombin generation in both the acute and prolonged streptokinase groups. In the latter group, the significantly higher levels of prothrombin fragment 1 + 2 were maintained throughout the 48 hours of the infusion of thrombolytic therapy. During the administration of placebo, there was an early decrease in thrombin activity, which is attributable to the concomitant use of heparin as previously described in patients with unstable angina and myocardial infarction. In the patients treated with acute streptokinase plus heparin, the decrease in thrombin activity was delayed and only became apparent after 24 hours. In the patients treated with prolonged streptokinase plus heparin this reduction was never observed, suggesting that the reduction in thrombin activity observed when patients receive placebo plus heparin is lost when concomitant streptokinase therapy is given.

Previous studies have already shown that the administration of thrombolytic therapy to patients with acute myocardial infarction induces the activation of the hemostatic mechanism that is demonstrated by an increase in thrombin generation and activity. Activation of the hemostatic mechanism has been observed using different thrombolytic agents, such as streptokinase, recombinant tissue plasminogen activator, anistreplase, or prourokinase, and direct comparisons of the different agents have shown that the level of activation is similar. Increased thrombin generation, expressed by high prothrombin fragment 1 + 2 plasma levels, has also been observed in a rather small series of patients receiving thrombolytic therapy for suspected acute myocardial infarction and in whom a diagnosis of unstable angina was subsequently made.

Activation of the hemostatic mechanism during thrombolysis has been attributed to the liberation of the thrombin bound to the thrombus or to the direct activation of coagulation factors by plasmin. Our finding of an activation of the hemostatic mechanism during thrombolytic therapy also in patients with unstable angina seems to indicate that the phenomenon occurs regardless of the amount and quality of intracoronary thrombus (which is occlusive and fibrin-rich in myocardial infarction, but subocclusive and platelet-rich in unstable angina). Moreover, the hypothesis of direct activation of coagulation is also supported by the fact that thrombolytic-induced activation of the hemostatic mechanism has also been observed in in vitro studies.

In patients with myocardial infarction, the increased thrombin generation observed during thrombolysis has been related to an increased risk of reocclusion. However, the beneficial effect obtained by opening a coronary occlusion that produces a life-threatening condition overwhelms the negative effects of the procoagulant action of thrombolytic therapy. In patients with unstable angina, the balance between the clot-dissolving and procoagulant actions of thrombolytic therapy may lead to a less favorable result. The beneficial effects of lysis are lower because, in most patients with unstable angina, the artery is already open. In addition, when present, thrombi appear to be predominantly composed of platelets rather than erythrocytes, which are considerably more resistant to lysis. Furthermore, the procoagulant action of thrombolytic therapy may be harmful at the site of a subtotal occlusion, because it may set in motion pathophysiological mechanisms favoring further thrombosis and therefore leading to total occlusion. It is therefore tempting to speculate that the more frequent progression toward myocardial infarction observed in patients with unstable angina receiving thrombolytic therapy may be partly related to the procoagulant action of the thrombolytic agent itself. Although the present investigation was not designed to test whether the thrombolytic-induced activation of the hemostatic mechanism might be associated with an adverse clinical outcome, it is worth noting that the only three patients who developed myocardial infarction were receiving thrombolytic therapy. Most interestingly, there was a clustering of ischemic episodes in the first 24 hours in the acute streptokinase group and in the first 48 hours in the prolonged streptokinase group (when hemostatic system function is actually
Fig 1. Distribution of adverse outcome events during the 72-hour study period. Each horizontal line represents a patient. (*), Myocardial infarction; (○), symptomatic ischemic episodes; (□), asymptomatic ischemic episodes.
increased); whereas in the placebo group the events were evenly distributed throughout the 72 hours of the study period. Finally, the fibrinopeptide A plasma levels were higher in the patients who subsequently developed a cardiac event, suggesting that the presence of increased hemostatic system function leading to increased thrombin activity is related to the subsequent clinical course.

As standard heparin treatment is incapable of blocking thrombolytic-induced activation of the hemostatic mechanism, further studies are needed to test whether more potent therapies with specific and direct antithrombins (such as hirudin), or with potent antiplatelet agents that bind to platelet GPIIb/IIIa receptors, may be more effective in inhibiting the activation of the hemostatic mechanism induced by thrombolytic agents in patients with unstable angina.

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REFERENCES

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