Persistent Generation of Thrombin After Acute Myocardial Infarction

Andrew Szczeklik, Jerzy Dropinski, Jadwiga Radwan, and Marek Krzanowski

During the acute phase of myocardial infarction, the generation of thrombin is reflected in the sudden rise of fibrinopeptide A (FPA) and the thrombin–antithrombin III (TAT) complex in blood. We have systematically determined the FPA and TAT plasma concentrations over a period of 14 days after acute myocardial infarction in 100 patients. Mean levels of both thrombin markers were the highest on admission, remained elevated over the following few days, and then gradually declined after day 5. Still, by the end of the first week two thirds of the patients had distinctly elevated TAT and FPA levels, and by the end of the second week such an abnormality was present in half of them. Continuous intravenous heparin infusion at a dose of 20,000 units/day, administered for 1 week to patients who had either received (n=21) or not received (n=17) streptokinase, led to a significant depression (p<0.05) of thrombin markers over the first 48 hours, an effect that did not persist over the subsequent days of treatment. In patients not assigned to heparin treatment, those in heart failure had significantly (p<0.05) higher mean TAT and FPA values on days 3, 5, and 7 compared with patients in whom heart failure was absent. Infarct extension, pulmonary embolism, and death were also associated with a rise in one or both thrombin markers, often preceding the onset of clinical symptoms. Thrombinogenesis was not accompanied by changes in mean plasma concentrations of prothrombin, antithrombin III, or α2-macroglobulin. It is suggested that thrombin was continuously released to the plasma from coronary or extracoronary thrombi undergoing lysis and subsequent rebuilding. Thus, thrombin generation that extends beyond the acute phase of myocardial infarction indicates an increased risk to the patients and might call for more anticoagulation, angioplasty, or the use of new antithrombotic agents. (Arteriosclerosis and Thrombosis 1992;12:548–553)

KEY WORDS • myocardial infarction • thrombin • heparin

Rupture of an atherosclerotic plaque initiates coagulation and leads to thrombosis of the coronary artery. This is the usual chain of events bringing about myocardial infarction. Such episodes are associated with the formation of thrombin. Local thrombinogenesis is reflected in the peripheral blood, where substantial amounts of fibrinopeptide A (FPA) can be detected. FPA is cleaved from fibrinogen by thrombin only. FPA is, therefore, a specific marker of thrombin generation in vivo. Another marker that has recently been introduced into clinical practice is a circulating complex of thrombin with its instant inhibitor, namely antithrombin III. It has been named the thrombin–antithrombin III (TAT) complex.

Over the past few years several authors have reported a marked elevation of the plasma FPA level during the acute stage of myocardial infarction. Very recently, a similar rise in TAT has also been described. All these studies were limited to the early phase of infarction and did not exceed a period of 48 hours. Only Johnsson et al extended their observations until the eighth day after infarction and noticed on that day an elevation of mean FPA levels in the seven patients studied.

Prolonged thrombinogenesis might predispose patients to have thromboembolic complications and might adversely affect the course of myocardial infarction. Therefore, we studied in a systematic way the levels of plasma FPA and TAT over a period of 2 weeks after acute myocardial infarction. We also quantified the major thrombin inhibitors in plasma.

Methods

Patients

We studied 100 consecutive patients (67 men and 33 women; average age, 60 years) who were admitted to the Intensive Care Unit because of acute myocardial infarction. Diagnosis was based on a typical history of chest pain, changes in the standard 12-lead electrocardiogram, and serial elevations of serum enzymes. Cardiac enzymes were measured every 6 hours during the first 24 hours, every 12 hours during the next 24 hours, and then once daily until their return to normal values. This procedure was repeated if the patient experienced a recurrence of the chest pain. The infarct was anterior in 57 patients and inferior in 43. Twenty-seven patients had had at least one myocardial infarction.

Twelve patients died in the hospital after all had been treated for at least 48 hours. Seven others had a sudden cardiac arrest within 24 hours after admission and were resuscitated. Thirty-four patients were in heart failure,
as evidenced by cardiogenic shock, pulmonary edema, or venostasis. Early postinfarction ischemia was diagnosed in 10 patients who had 1) anginal pain at rest or at minimal exercise during the hospital stay 24 hours or more after definite infarction and 2) electrocardiographic ischemic changes, as described by Bosch et al. An extension of infarction during hospitalization, as diagnosed by the same criteria that were used for the initial infarct, occurred in eight patients. Five of eight patients with infarct extension had experienced early postinfarction ischemia, and three had not.

Treatment
All patients were treated with bed rest, oxygen by nasal cannula, and various drugs as clinically required. Depending on the specific therapy received, the patients were divided into three groups: 1) no streptokinase (SK) and no heparin (41 men and 21 women; average age, 61 years); 2) SK and heparin (14 men and seven women; average age, 57 years). These patients were admitted to the Intensive Care Unit within 4 hours of the onset of chest pain. SK was given at a dose of 1,500,000 units over 30–60 minutes. Heparin was then administered intravenously as a bolus at a dose of 5,000 units, which was followed by an intravenous infusion of 20,000 units/day, given by an infusion pump, for 1 week; and 3) heparin only (12 men and five women; average age, 58 years). These patients did not receive SK but were given heparin as a continuous intravenous infusion at a dose of 20,000 units/day for 1 week. The infusion was started within 24 hours after admission.

Blood Sampling and Analytical Methods
Blood was obtained after admission and then on days 2, 3, 5, 7, and 14. In 10 patients blood was also sampled before and 10, 20, and 30 minutes after SK infusion. Additional sampling was performed between days 8 and 14 for patients with clinical complications. After the first 2–3 ml of blood was discarded, the samples were collected into tubes containing the appropriate anticoagulant mixtures, processed immediately, and frozen at −20°C. Estimations were then performed within a month.

FPA was measured in platelet-poor plasma by use of commercial radioimmunoassay kits (Mallinckrodt, Inc., Dietzenbach, FRG). The procedure outlined by the manufacturer was followed, and the anticoagulant mixture provided in the kit was used. Results are expressed as averages of paired samples run in duplicate. TAT complexes were measured in duplicate aliquots of citrated plasma with an enzyme-linked immunosorbent assay technique based on the sandwich principle (Enzygnost TAT, Behringwerke, Marburg, FRG). In 20 healthy volunteers matched for age (13 men and seven women), the TAT concentration was 2.1±0.8 ng/ml and the FPA level was 2.4±1.2 ng/ml (mean±SD). These values were somewhat higher compared with the levels referred to as normal by the test manufacturers but were well within the range obtained by other authors who have used the same kits. Our interassay coefficients of variation were 5% for TAT and 9% for FPA. Levels exceeding two standard deviations of the mean were considered abnormally elevated (for TAT, >4 ng/ml and for FPA, >5 ng/ml).

In 30 consecutive patients of the 62 not assigned to SK or heparin treatment, we measured plasma concentrations of prothrombin and the two major thrombin inhibitors to evaluate whether their possible fluctuations would explain the changes in thrombin markers. Prothrombin was measured by a kinetic colorimetric method (Faktor II, Boehringer Mannheim GmbH, Vienna, Austria), and anti-thrombin III (Bio-Merieux, Charbonnières les Bains, France) and α1-macroglobulin (Behringwerke, Marburg, FRG) were measured by radial immunodiffusion. The samples were collected on the same days as those for TAT and FPA concentrations.

Statistical Evaluation
Statistical analysis was carried out on a personal computer using Complete Statistical System (StatSoft, Inc.) software.

As noted by other authors, in patients with myocardial infarction the thrombin markers showed a skewness toward high values. The data were, therefore, logarithmically transformed, and statistical analysis was performed using a two-tailed t test. Results are reported as mean±SEM; geometric mean values are added in the tables.

Results
On admission, TAT was elevated in 90 of 100 patients and FPA was elevated in 95 of 100 patients with acute myocardial infarction. Only in two patients were both FPA and TAT within the normal limits. Mean TAT and FPA levels were at their highest levels on day 1 (Table 1). Over the following days both TAT and FPA remained elevated but gradually declined after day 5. Still, on the seventh day, 63% of all patients had an elevated TAT level and 67% had an elevated FPA level. On day 14, TAT was raised in 57% of patients and FPA in 51%. Peak creatine phosphokinase (CPK) and peak serum glutamic-oxaloacetic transaminase (SGOT) levels showed a positive correlation with both TAT and FPA values on days 1 and 7 in a group of patients who received neither SK nor heparin (i.e., peak CPK versus TAT, r=0.67, p=0.01 on day 1 and r=0.49, p=0.06 on day 7; SGOT versus TAT, r=0.48, p=0.01 on day 1 and r=0.65, p<0.01 on day 7). Such correlations were absent in patients who were treated with SK and heparin.

In individual cases the magnitude of the response of TAT and FPA was far from parallel. The correlation coefficients estimated for all 100 patients were very low on days 1, 2, and 3 (r<0.15, p>0.3) but were improved on day 5 (r=0.33, p=0.06), day 7 (r=0.26, p=0.11), and day 14 (r=0.37, p=0.07).

Within 10–20 minutes after completion of the SK infusion, TAT and FPA values increased by two to seven times in eight of nine patients studied. When heparin was then given as a bolus of 5,000 units followed by an intravenous infusion, within 1 hour both thrombin markers were reduced to about 20% of post-SK values. Continuous intravenous heparin infusion was often associated with a depression of raised thrombin marker values. Mean TAT and FPA values were significantly lower (p<0.05) only on days 2 or 3 in patients assigned to heparin treatment (irrespective of their receipt of SK treatment) compared with nonheparinized patients (Ta-
Table 1. Plasma Levels of Thrombin-Antithrombin III Complex and Fibrinopeptide A in All Patients and in Subgroups Assigned to Different Treatments

<table>
<thead>
<tr>
<th>Patient group/thrombin marker</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td>35.7±4.7</td>
<td>23.8±6.4</td>
<td>30.0±5.1</td>
<td>31.4±3.8</td>
<td>25.1±4.0</td>
<td>18.8±3.6</td>
</tr>
<tr>
<td>FPA</td>
<td>17.1</td>
<td>12.5</td>
<td>13.7</td>
<td>14.5</td>
<td>12.2</td>
<td>9.6</td>
</tr>
<tr>
<td>SK and heparin (n=62)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td>34.1±6.9</td>
<td>30.5±9.7</td>
<td>27.2±7.2</td>
<td>33.2±7.8</td>
<td>20.2±4.6</td>
<td>18.6±5.1</td>
</tr>
<tr>
<td>FPA</td>
<td>15.4</td>
<td>13.5</td>
<td>11.3</td>
<td>15.0</td>
<td>10.1</td>
<td>9.4</td>
</tr>
<tr>
<td>Heparin only (n=17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAT</td>
<td>42.3±12.7</td>
<td>10.1±4.3*</td>
<td>18.4±10.9</td>
<td>31.2±17.2</td>
<td>24.1±11.0</td>
<td>11.0±13.1</td>
</tr>
<tr>
<td>FPA</td>
<td>28.0±11.0</td>
<td>35.0±24.0</td>
<td>8.9±4.4*</td>
<td>20.2±10.6</td>
<td>23.6±20.5</td>
<td>18.2±24.0</td>
</tr>
</tbody>
</table>

Table 2. Plasma Levels of Thrombin-Antithrombin III Complex and Fibrinopeptide A in Patients Who Did Not Receive Streptokinase or Heparin

<table>
<thead>
<tr>
<th>Thrombin marker/heart failure</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>34.8±9.2</td>
<td>36.0±21.0</td>
<td>47.9±13.0</td>
<td>61.1±10.2</td>
<td>33.2±11.0</td>
<td>32.2±21.0</td>
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<tr>
<td>-</td>
<td>16.0</td>
<td>16.4</td>
<td>31.2</td>
<td>49.5</td>
<td>15.1</td>
<td>13.4</td>
</tr>
<tr>
<td>FPA</td>
<td>33.7±9.6</td>
<td>22.1±16.0</td>
<td>7.5±1.5*</td>
<td>16.1±4.1*</td>
<td>20.2±4.0*</td>
<td>14.8±4.5</td>
</tr>
<tr>
<td>+</td>
<td>15.1</td>
<td>8.8</td>
<td>2.0</td>
<td>6.1</td>
<td>12.0</td>
<td>10.3</td>
</tr>
<tr>
<td>-</td>
<td>30.1</td>
<td>29.4</td>
<td>29.2</td>
<td>51.4</td>
<td>58.2</td>
<td>12.4</td>
</tr>
<tr>
<td>FPA</td>
<td>59.4±15.2</td>
<td>56.2±14.7</td>
<td>24.1±10.4*</td>
<td>30.7±14.2*</td>
<td>33.2±13.8*</td>
<td>12.6±9.9</td>
</tr>
</tbody>
</table>

Patients could be clinically differentiated depending on the presence (+; n=16) or absence (−; n=33) of heart failure. Geometric means are presented under arithmetic mean±SEM.

TAT, thrombin-antithrombin III complex; FPA, fibrinopeptide A.

*Significant differences (p<0.05, unpaired t test) between patients with and without heart failure.
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Cardiac arrest on admission did not seem to affect the subsequent course of thrombin marker levels. A high persistent rise in TAT and FPA values heralded a poor prognosis. Such a pattern was present in 10 of 12 patients who died between the fourth and the 36th day (average, 12th day) (Figure 2). Their deaths were preceded by progressive heart failure. Six had repeated bouts of arrhythmias, five had reinfarction, and four had pulmonary emboli.

Infarct extension was diagnosed in eight patients. In all of them, reinfarction coincided with a marked rise in TAT, and in seven of eight, with a rise in FPA. Three of these subjects developed reinfarction while they were receiving continuous intravenous heparin (Figure 3).

Mean concentrations of plasma prothrombin, antithrombin III, and α2-macroglobulin showed no significant changes (p>0.05, paired t test) during the first 2 weeks of infarction in 30 consecutive patients who received neither SK nor heparin (data not shown).

Discussion

Our observations indicate that the generation of thrombin that accompanies myocardial infarction is a long-lasting process that extends well beyond the acute phase of the disease. By the end of the first week, two thirds of patients had both thrombin markers that were distinctly raised, and by the end of the second week such an abnormality was present in half of them.

These observations cannot be explained by either an increased production of prothrombin or alterations in the synthesis of the major thrombin inhibitors. In patients not assigned to SK or heparin therapy, mean plasma values of prothrombin, antithrombin III, and α2-macroglobulin remained unchanged over a period of 14 days. Rather, we suspect that thrombin was released to plasma mainly from coronary thrombi. Both in vitro and in vivo, thrombin becomes absorbed to fibrin at the time of clot formation. In acute coronary syndromes, the clot usually anchors on ruptured atherosclerotic plaques that have active matrix-bound thrombin and are platelet rich. Spontaneous or drug-induced thrombolysis is often followed by platelet accumulation and rapid rebuilding of the thrombus. Such an ongoing process could account for the persistent generation of thrombin that we observed in most of our patients. It is also interesting to note that in our patients the second peak of thrombin markers coincided with increased aggregability in circulating platelets, as previously described. Several authors have reported a distinct hyperreactivity of platelets 4–7 days after myocardial infarction.

Until now, FPA and TAT had not been simultaneously determined in patients with myocardial infarction. In vitro, after the addition of thrombin to citrated plasma, TAT and FPA levels correlate closely. In vivo, less clear-cut results were obtained. In our study the correlation between the two markers was rather weak, possibly because of the differences in half-life and clearance rates of the markers and their different redistributions between the extravascular and the intravascular space, as well as sample collection and processing.
Persistent generation of thrombin was not easily counteracted by heparin. The short-term effects of heparin treatment were evident: both FPA and TAT levels became markedly depressed, and this effect lasted until the second day. A similar early suppression of plasma FPA was observed by Mombelli et al in patients who received a continuous infusion of heparin at a dose of 20,000 IU/day for 48 hours after infarction. However, when we extended the infusion time beyond 48 hours, the effects became far less evident. Despite retardation of blood clotting, as reflected by a prolongation of the activated partial thromboplastin time, mean TAT and FPA values were not significantly lower after 4–5 days of heparin therapy compared with nonheparinized patients. Moreover, quite a few patients while on continuous intravenous heparin treatment experienced a sudden rise in TAT and/or FPA levels followed by pulmonary emboli, infarct extension, or aggravation of heart failure. Three reasons might explain the limited antithrombin action of heparin. First, a residual thrombus contains active thrombin bound to fibrin, which is thus poorly accessible to the large heparin–antithrombin III complex. Second, a platelet-rich arterial thrombus releases large amounts of platelet factor 4, which inhibits the action of heparin. Third, the fibrin II monomer, which is formed by the action of thrombin on fibrinogen, also inhibits heparin action. Finally, recent experimental evidence suggests that heparin in medium–high therapeutic dosages that prevent blood coagulation and prolong partial thromboplastin time is unable to inhibit local thrombin generation at the site of severe vessel wall injury.

It was the clinical course rather than the assigned treatment that determined the behavior of the thrombin markers in our patients. Indeed, heart failure proved to be a discriminating factor. Patients in cardiogenic shock, pulmonary edema, or venostasis were characterized by high levels of thrombin markers that remained elevated for several days, even when the clinical symptoms of heart failure had receded. In these patients the course of thrombin markers was strikingly different from those who were not in heart failure at the time of thrombolysis. However, all patients, but not in the latter, in whom the suppression of thrombin generation by a continuous heparin infusion was largely ineffective.

Several factors could account for the extended elevation of thrombin markers in patients with heart failure, the first being an extension of myocardial infarction. On day 7 there was a positive correlation between concentrations of CPK and both TAT and FPA. Second, our observations point to still another clinical interest in TAT and FPA assessment. In our patients, persistent thrombin generation extending beyond the acute phase of infarction was associated with heart failure, infarct extension, pulmonary emboli, and death. In individual patients, a rise in the TAT and/or FPA level preceded a clinical presentation of complications. Thus, high levels of thrombin markers indicate an increased risk to a patient with myocardial infarction. Persistent, high FPA and/or TAT plasma levels, despite heparin anticoagulation, point to ongoing thrombin generation that may necessitate more anticoagulation, increased antiplatelet treatment, angioplasty, or, in the future, use of new antithrombotic drugs.

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