DIAGNOSTIC METHODS
CORONARY THROMBOSIS

Effect of heparin on plasma fibrinopeptide A in patients with acute myocardial infarction

G. Mombelli, M.D., V. Im Hof, M.D., A. Haeberli, Ph.D., and P.W. Straub, M.D.

ABSTRACT  The plasma level of fibrinopeptide A (fpA) was used as an index of thrombin action on fibrinogen in order to investigate the rates of fibrin formation and the effect of heparin on thrombin in patients with acute myocardial infarction. The fpA levels measured on admission in 19 patients with acute myocardial infarction ranged from 1.7 to 12.4 ng/ml and were elevated (>2.5 ng/ml) in 16 patients. A loading dose of 5000 IU of heparin resulted in a significant decrease within 20 min of the mean fpA level (from 5.1 to 2.2 ng/ml; p < .001) and in an fpA normalization in five of 16 patients. During the following continuous infusion of 20,000 IU of heparin per day, the mean fpA levels measured on day 0, 1, and 2 were 3.0, 3.2, and 3.4 ng/ml, respectively, with 16 of 46 fpA values within the normal range. In 10 additional patients, the effect of higher concentrations of heparin and the consequences of stopping heparin infusion were studied. An additional 5000 IU of heparin injected intravenously during continuous infusion of 20,000 IU of heparin per day resulted in a substantial decrease of the plasma fpA level in three of 10 measurements. The stopping of heparin infusion led to an impressive increase of the mean fpA level (from 3.1 to 12.9 ng/ml; p < .001) within 2 hr. These data demonstrate increased fibrin formation in patients with acute myocardial infarction and neutralization of thrombin in vivo by heparin. They also suggest that heparin doses higher than those conventionally used may be required to fully inhibit fibrin formation and that additional thrombin may be generated after cessation of heparin infusion.


THE ASSOCIATION between acute myocardial infarction and thromboembolic phenomena has long been recognized. Whereas coronary thrombosis may be a significant factor in the genesis of myocardial infarction,1 arterial and venous thromboembolisms often complicate the course of the disease.2,3 Nevertheless, the usefulness of heparin in treating patients with acute myocardial infarction remains controversial.

Several recent studies4-6 have indicated that the plasma level of fibrinopeptide A (fpA), a specific product of fibrinogen cleavage by thrombin, is a useful index of fibrin formation in vivo. Since heparin acts by inhibiting thrombin action on fibrinogen, the study of the effect of heparin on fpA should provide direct information concerning the effectiveness of heparin in preventing fibrin formation in vivo.

In this study, the plasma level of fpA was measured in patients with acute myocardial infarction to investigate the rates of fibrin formation and the thrombin-neutralizing effect of heparin in this disorder.

Methods

Patients and care. A total of 29 patients admitted to the coronary care unit of the University of Bern because of acute myocardial infarction were studied. Criteria for inclusion in the study were: (1) typical chest pain within 32 hr of admission, (2) pathologic Q waves and/or ST-T segment changes in 12-lead electrocardiogram, (3) presence of elevated creatine kinase levels, (4) absence of thromboembolic disorders, (5) absence of comorbid factors known to elevate fpA (malignancy, sepsis, nephritis, collagen disorder), (6) absence of anticoagulant drugs within the last month, and (7) absence of bleeding disorders. The clinical data of the patients are summarized in table 1.

All patients were treated with bed rest, oxygen by nasal cannula, and by various drugs as clinically required. On admission, a loading dose of 5000 IU (58 to 82 IU/kg) of heparin (Liquemin; Hofmann–La Roche, Basle) was administered intravenously in a bolus form. This was followed by an intravenous infusion of 20,000 IU (230 to 330 IU/kg) of heparin a day given continuously by an infusion pump. The heparin treatment was continued for at least 2 days.

Protocol. The effect of the loading dose and of the subsequent continuous infusion of heparin on the plasma fpA level was studied in the first 19 patients. In these patients, blood samples were taken immediately before and after 20 min after the initial bolus of heparin, as well as 6 to 8 hr (day 0), 1 day (day 1), and 2 days (day 2) after the start of the heparin infusion.

In the next 10 patients, the effect of additional heparin injected during continuous infusion of the anticoagulant and the con-
sequence of stopping heparin infusion on the plasma fpA level was investigated as follows: In the first five of these patients, a second and third bolus of heparin (5000 IU, 59 to 72 IU/kg) were given on days 1 and 2 of heparin infusion, and blood samples were taken immediately before and 20 min after the bolus. In the last five patients, heparin infusion was stopped during 2 hr on days 1 and 2, and blood samples were taken immediately before and 2 hr after cessation of heparin infusion.

Coagulation tests. Blood samples were taken by careful venipuncture through an 18-gauge needle. All collections were done by the same investigator. For fpA measurements, 4.5 ml of blood was collected into a plastic syringe containing 500 U of heparin and 500 U of aprotinin, dissolved in 0.5 ml of physiologic saline. The blood samples were then centrifuged at 3000 rpm at 4°C for 10 min; the plasma samples were stored at −25°C. They were then dialyzed to separate fpA from plasma and were assayed for the fpA. The radioimmunoassay for fpA was performed as previously reported,7 except that instead of charcoal, an immobilized goat antirabbit immunoglobulin (Immuno beads, Bio Rad Laboratory) was used for separation of free and bound fpA. With this method the fpA level measured in 15 healthy persons was 1.9 ± 0.5 ng/mL.

Other clotting tests were performed by the following methods: fibrinogen concentration by the method of Clauss,8 antithrombin III by an amidolytic assay with Chromozym TH (Boehringer; Mannheim, West Germany), heparin concentration with an amidolytic assay adding excess antithrombin III and factor Xa, and measuring residual amounts of factor Xa by the substrate S-2222 (Coatest, Kabi Diagnostica).

Data analysis. The measured levels of fpA showed a skewness toward high values, and logarithmic transformation was required to obtain a normal distribution. Statistical analyses were therefore performed with fpA data logarithmically transformed, and mean fpA levels are expressed as geometric means with standard deviations of the logarithmically transformed data. Evaluation of relationships among different parameters was performed by linear least squares regression analysis. Statistical significance of changes in the same parameter was evaluated with Student’s t test for either paired or independent samples.

Results

Levels of fpA on admission and effect of the initial bolus of heparin. The fpA levels measured on admission in 19 patients with confirmed infarction ranged from 1.7 to 12.4 ng/ml (5.1 ± 1.8, mean ± SD) and were elevated (>2.5 ng/ml) in 16 patients. Elevated fpA values were found in eight of 10 patients studied within 6 hr and in eight of nine patients studied 6 to 32 hr after the onset of symptoms; no significant correlation between the fpA level and the duration of symptoms was found. The 13 patients with peak creatine kinase levels above 500 IU showed higher mean fpA values (5.4 ± 2.0 ng/ml) compared with the values in the six patients with peak creatine kinase levels below 500 IU (4.5 ± 1.5 ng/ml). However, the difference was not statistically significant, and there was again no significant correlation between the fpA level on admission and the peak creatine kinase level in plasma.

The effect of the loading dose of 5000 IU of heparin is shown in figure 1. The intravenous bolus of heparin

![Figure 1](http://circ.ahajournals.org/)

**FIGURE 1.** Levels of fpA before and 20 min after initial bolus of heparin (5000 IU).
resulted (within 20 min) in a significant fall of the fpA level from 5.1 ± 1.8 to 2.2 ± 2.0 ng/ml (p < .001) and in an fpA normalization in five of 16 patients. The heparin levels measured in plasma 20 min after injection ranged from 0.3 to 1.1 IU/ml (0.77 ± 0.2).

Levels of fpA, heparin, fibrinogen, and antithrombin III under continuous heparin infusion. Table 2 summarizes the mean plasma levels of fpA, heparin, fibrinogen, and antithrombin III measured in the 19 patients on day 0, 1, and 2 of continuous infusion of heparin (20,000 IU/day). The fpA values measured under heparin treatment were significantly lower than those observed in patients on admission (5.1 ± 1.8 vs 3.2 ± 2.3 ng/ml; of p < .01). The single levels were, however, elevated in 30 of 46 (65%) samples. Patients with cardiogenic shock or ventricular tachycardia/fibrillation showed a higher mean fpA value (4.1 ± 2.2 ng/ml) compared with that of patients without circulatory disturbances (3.1 ± 2.3 ng/ml), but the difference was not statistically significant. None of the 19 patients presented clinical signs of arterial or venous thromboembolism or hemorrhagic complications.

During the first 2 days after admission, the mean plasma fibrinogen level increased from 3.2 to 5.0 mg/ml (p < .01), whereas the antithrombin III level decreased from 80.5% to 67.5% (p < .01). Although the daily dose of heparin remained unchanged, there was a continuous decrease of the heparin concentration in plasma, with a mean level of 0.36 IU/ml on day 0 and 0.15 IU/ml on day 2 (p < .02). When the fpA values measured on admission, after the initial bolus of heparin, and during continuous heparin infusion were related to the corresponding heparin levels, a significant negative relationship could be established between the two parameters (n = 88, r = −.359, p < .01) (figure 2). At heparin levels above 0.3 IU/ml, the fpA values were significantly higher in patients with ventricular tachycardia/fibrillation or cardiogenic shock than in those without circulatory disturbances (6.3 ± 1.6 vs 2.2 ± 2.2 ng/ml; p < .01). No relationship was found between the fpA level and the level of fibrinogen or antithrombin III.

Effect of additional heparin injections on fpA. In five other patients with acute myocardial infarction, the intravenous injection of an additional 5000 IU of heparin on day 1 and 2 of continuous heparin infusion (20,000 IU/day) lowered the mean fpA level from 6.2 ± 2.0 before to 3.7 ± 1.8 ng/ml 20 min after the bolus of heparin was administered (p > .05) (figure 3). The fpA level dropped substantially in the three patients who had highly elevated fpA levels before the bolus, reaching normal concentrations in two of them. In the seven patients who had only moderate fpA elevations during continuous heparin infusion, the bolus did not lead to a significant change of the fpA in plasma.

Consequence of stopping heparin infusion on fpA. In five

### TABLE 2
Mean levels (±SD) measured in patients with myocardial infarction during continuous heparin infusion (n = 19)

<table>
<thead>
<tr>
<th>Day after admission</th>
<th>fpA (mg/ml)</th>
<th>Heparin (IU/ml)</th>
<th>Antithrombin III (%)</th>
<th>Fibrinogen (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0 ± 2.9</td>
<td>0.36 ± 0.24</td>
<td>80.5 ± 10.5</td>
<td>3.2 ± 0.9</td>
</tr>
<tr>
<td>1</td>
<td>3.2 ± 1.8</td>
<td>0.19 ± 0.16</td>
<td>75.3 ± 6.2</td>
<td>4.0 ± 1.0</td>
</tr>
<tr>
<td>2</td>
<td>3.4 ± 2.2</td>
<td>0.15 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67.5 ± 11.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.0 ± 1.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values of day 0 were determined on blood samples taken 6 to 8 hr after beginning of continuous heparin treatment.

<sup>b</sup>p < .02 as compared with day 0.

<sup>c</sup>p < .01 as compared with day 0.

![Figure 2](image_url)

**FIGURE 2.** Relationship between fpA and heparin levels in 19 patients with acute myocardial infarction. Included are the values measured before and 20 min after the initial bolus of heparin as well as those measured on days 0 to 2 of continuous heparin infusion. Open circle = values for patients without circulatory disturbances; closed circle = values measured during and/or after ventricular tachycardia/fibrillation or cardiogenic shock.
found in our patients should mostly reflect a short-term event related to infarction. The lack of correlation between peak creatine kinase and fpA levels in plasma suggests that the rise in fpA levels was not a simple consequence of myocardial damage. Moreover, the impressive decline in fpA levels observed after the injection of heparin, a thrombin inhibitor, strongly implies that the fpA was generated by the intravascular action of thrombin on fibrinogen. These findings confirm previous observations indicating thrombin activation and increased fibrin formation in the early phase of myocardial infarction. In this setting, intravascular fibrin formation could reflect thrombotic coronary occlusion, parietal thrombosis over a transmural infarction, or early development of venous thrombi. Coronary thrombosis has been found in 87% of the patients with myocardial infarction, whereas the incidence of left ventricular thrombi and venous thrombi has been 17% and 37%, respectively.

In patients with infarction not receiving heparin, the rise in fpA level has been reported to remain pronounced during several days after the onset of symp-

![Figure 3](http://circ.ahajournals.org/)

**FIGURE 3.** Levels of fpA before and after a second and third bolus of heparin (5000 IU) administered without stopping constant heparin infusion (20,000 IU/day).

additional patients with acute myocardial infarction, heparin infusion was stopped for 2 hr on days 1 and 2, and the corresponding changes in plasma fpA levels are shown in figure 4. Decline in plasma heparin concentration resulted in an impressive increase in the mean plasma fpA level, which rose from 3.2 ± 2.4 immediately before to 12.9 ± 3.3 ng/ml 2 hr after heparin infusion was stopped (p < .001).

**Discussion**

We found elevated plasma fpA levels in eight of 10 patients admitted with acute myocardial infarction who were studied within 6 hr and in eight of nine who were studied within 6 to 32 hr after the onset of symptoms. In a previous study, the plasma fpA level was found to be normal in patients with stable coronary heart disease, except for in a few patients with left ventricular thrombosis. Therefore, the fpA elevation

![Figure 4](http://circ.ahajournals.org/)

**FIGURE 4.** Levels of fpA before and after stopping constant heparin infusion (20,000 IU/day). Zero point indicates time at which heparin infusion was stopped.
In our study, continuous heparin infusion led to a significant decrease of the mean fpA value, while the single values remained mildly to markedly elevated in several patients. In some patients, particularly in those with circulatory disturbances, elevated fpA levels were found in spite of high concentrations of heparin. This observation suggests either failure of heparin to inhibit fpA generation or excessive heparin neutralizing activity in plasma. In other patients, however, the fpA elevation was associated with low concentrations of heparin. Moreover, the administration of additional heparin during continuous heparin infusion resulted in a substantial decrease in fpA in the patients with high fpA levels. These data strongly suggest that 20,000 IU of heparin per day was inadequate to prevent intravascular fibrin formation in some patients. This conclusion is corroborated by a recent study, which reported evidence of left ventricular thrombosis in patients with infarction who were receiving the same dose of heparin.

In acute infarction, the requirement for high doses of heparin may result from several factors. First, we observed a progressive decrease of the level of heparin over the first 2 days after admission in the patients, suggesting that there was a progressive increase in heparin clearance after infarction. The increased rate of heparin clearance may reflect binding of heparin to an antithrombin-thrombin complex on the surface of thrombi, as has been suggested to occur in patients with pulmonary embolism. Second, the antithrombotic effect of heparin may have been impaired by the observed increase of the fibrinogen level or decrease of the antithrombin III level. However, the lack of correlation between the levels of these two proteins and the plasma fpA level does not support this hypothesis. Finally, heparin could have been inhibited by platelet factor 4 or other heparin-neutralizing proteins such as α1-acid glycoprotein and histidine-rich glycoprotein. These possibilities were not investigated in our patients.

In most instances, the injection of additional heparin during continuous infusion of the anticoagulant did not normalize fpA. This has already been reported in patients with malignancy or sepsis. It indicates that fpA is not produced by cleavage of fibrinogen at sites accessible to heparin and implicates either extravascular deposition of fibrin or fpA release by mechanisms other than thrombin. In acute infarction, extravascular generation of fpA may result from fibrinous pericarditis or from fibrin deposition within the necrotic tissue. Alternatively, the fpA may be generated by proteases of leucocytes entering the infarcted myocardium.

An unexpected finding was the impressive increase in plasma fpA levels after heparin infusion was stopped. The mechanism of the rebound in fpA levels is uncertain. It suggests that additional thrombin was generated as heparin was cleared from plasma. It may be speculated that thrombin was released from heparin-antithrombin-thrombin complex or from fibrin. Whatever the mechanism, the observation will have important therapeutic implications, and further studies are being carried out to define the consequences of stopping heparin treatment in patients with this disease.

At the present time, the usefulness of heparin during acute myocardial infarction is controversial, and there is no uniformity concerning the dosage and route of administration of the anticoagulant. The present data indicate that heparin reduces fibrin formation in patients with acute myocardial infarction, but suggest that heparin doses higher than conventionally used may be required to fully inhibit thrombin. This conclusion agrees with a recent clinical observation indicating that even "full" heparin doses may fail to prevent parietal thrombosis. Whether the benefit of high heparin doses is worth the risk of drug-induced hemorrhage in patients with acute infarction remains to be tested in clinical studies.

References

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Circulation. 1984;69:684-689
doi: 10.1161/01.CIR.69.4.684

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

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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