Fibrinopeptide A: a marker of acute coronary thrombosis*

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ABSTRACT To determine whether coronary thrombosis in vivo is reflected by elevations in levels of fibrinopeptide A (FPA) in plasma, we sequentially characterized plasma FPA levels associated with evolving infarction in patients admitted to the cardiac care unit early after the onset of symptoms, in patients with transmural infarction admitted later, and in patients with nontransmural infarction. Studies were also performed in patients in whom the diagnosis of infarction was suspected but subsequently excluded. FPA values were significantly higher in patients with transmural infarction (42.3 ± 11.2 ng/ml [mean ± SEM], n = 53) compared with those in patients with nontransmural infarction (4.8 ± 1.6 ng/ml, n = 17) or with those in patients in whom infarction was subsequently excluded as a diagnosis (3.5 ± 0.6 ng/ml, n = 17, p < .01 for both). Elevations in FPA level were greatest in patients with transmural infarction from whom samples were obtained soon after the onset of symptoms. Thus, in 39 patients from whom samples were obtained within 10 hr after the onset of symptoms, FPA levels were significantly higher than in 14 patients from whom samples were obtained initially more than 10 hr after the onset of symptoms (55.5 ± 14.7 vs 4.9 ± 1.4 ng/ml, p < .01). In 30 of the 39 patients with evolving transmural infarction from whom samples were obtained within the first 10 hr after the onset of symptoms, the level of FPA was greater than 8 ng/ml. In contrast, FPA level was greater than 8 ng/ml in only two of the 14 patients from whom initial samples were obtained more than 10 hr after the onset of symptoms. In patients admitted early after transmural infarction from whom samples were obtained sequentially (n = 8), FPA values declined consistently during the 24 hr sampling interval. Thus, an elevated FPA level appears to be a criterion of acute coronary thrombosis.


CORONARY THROMBOSIS is often associated with acute myocardial infarction. It appears to initiate or perpetuate the process frequently.1,2 Although factors precipitating coronary thrombosis have not yet been elucidated fully, activation of thrombin is common to thrombosis in general. Fibrinopeptide A (FPA) is a product of proteolysis by thrombin of fibrinogen.3 Thus, elevation of FPA levels in plasma is seen in association with disorders such as disseminated intravascular coagulation, deep venous thrombosis, arterial thrombosis, and malignancy.4,6 Elevations have also been associated with coronary artery disease, but surprisingly the magnitude of the elevation has not been reported to differ between patients with acute myocardial infarction, and presumably a higher likelihood of acute coronary thrombosis, and those with chronic coronary artery disease.7–12 Because conversion of fibrinogen to fibrin is particularly rapid early during the course of thrombosis13 and because the half-life of FPA in plasma is so short (3 to 5 min),1 it seemed likely that the elevation of FPA level associated with acute coronary thrombosis might be identifiable if samples were obtained very early after the onset of symptoms of transmural infarction. Accordingly, we compared levels of FPA in plasma samples from patients admitted with transmural infarction early after the onset of symptoms with values in similar patients admitted later, in patients with nontransmural infarction, and in patients in whom a diagnosis of infarction was suspected but was subsequently excluded. Our data provide an explanation for the results of previous studies and suggest an important role for measurement of FPA levels in patients with acute myocardial infarction.
Methods

This study was approved by the Human Studies Committee of the Washington University School of Medicine. Patients admitted to the cardiac care unit of Barnes Hospital with a diagnosis of suspected myocardial infarction without severely limited venous access, known malignancy, disorders of hemostasis, or chronic renal failure and without medical regimens that included anticoagulants were evaluated. Plasma samples for determination of FPA levels were obtained immediately after admission concurrently with those for assay of MB-creatinine kinase (CK) activity to confirm or exclude the presence of infarction. In selected patients additional samples were obtained three times daily at 8 hr intervals for FPA assays. The diagnosis of myocardial infarction was corroborated by elevation of MB-CK level (greater than 11 IU/ml) and characteristic serial electrocardiographic changes. Transmural infarction was considered to be present if new Q waves (greater than 40 msec) appeared. In their absence, infarction was considered to be nontransmural.

A subset of patients required emergency coronary angiography for evaluation of their suitability for treatment with fibrinolytic agents. Coronary artery thrombus was considered to be present only if complete thrombotic occlusion of the infarct-related artery was demonstrable angiographically. All patients were evaluated to determine whether or not thrombotic events were occurring throughout the sampling interval, to define all medications taken, and to record major clinical events. Patients with anterior infarction were studied with two-dimensional echocardiography within 24 hr of admission to identify left ventricular mural thrombi.

Blood samples. Samples were drawn by venipuncture by specially trained technicians who were evaluated by use of quality control procedures before and during the study. The first 2 to 3 ml of blood was discarded and the subsequent sample was collected with uninterrupted flow into precooled tubes (4°C) containing EDTA and aprotinin (provided by Mallinkrodt, Inc., St. Louis), cooled to 4°C immediately, and promptly centrifuged at 1000 g. Plasma was separated immediately and fast frozen at −20°C for no more than 24 hr and subsequently at −70°C before assay. A log was kept to identify any difficulty encountered with venipuncture, acquisition of samples, or processing.

Assay procedures. FPA level was measured by radioimmunoassay in plasma samples after treatment with bentonite to absorb fibrinogen. The assay was standardized for measurement of FPA levels of from 1.0 to 1000 ng/ml. Plasma was separated immediately and fast frozen at −20°C for no more than 24 hr and subsequently at −70°C before assay. A log was kept to identify any difficulty encountered with venipuncture, acquisition of samples, or processing. The upper limit of normal was taken as 2.0 ng/ml. Samples with FPA levels of greater than 40 ng/ml were diluted with a standard plasma with an undetectable amount of FPA by a procedure validated to provide accurate values for samples with defined concentrations of FPA up to 8000 ng/ml. Results were expressed as averages of paired samples run in duplicate. The mean coefficient of variation was 5.66 ± 0.73% (mean ± SEM).

MB-CK was assayed with the glass-bead absorption method previously described in samples drawn into tubes containing ethyleneglycol bis-n-n tetracetic acid (8 nM final concentration) and mercaptoethanol (12 nM final concentration). Samples were centrifuged immediately, refrigerated, and assayed within 10 hr. When the level of MB-CK was elevated in the first sample, back-extrapolation of the time-activity curve was employed to identify the time of onset of infarction, as previously described. Infarct size was estimated from serial plasma MB-CK values.

Statistical analysis. Raw data are reported as mean ± SEM. A log transformation was used to normalize FPA values, the intervals between the onset of symptoms and the time of initial sampling, and the values for infarct size for statistical analysis. Data were tested by three-way analysis of variance, t tests, and Pearson correlation coefficients obtained with the SAS statistical program (SAS Institute, Cary, NC) and computer facilities of the Washington University Biomedical Computing Laboratory.

Results

In 83 patients with suspected acute myocardial infarction FPA was assayed in samples obtained soon after admission. Samples from five patients were not analyzed after being excluded prospectively because of problems associated with sampling. The mean age of the patients studied was 59.9 ± 1.3 years (n = 77). Twenty-seven of the patients were women and 50 were men (table 1).

Elevated MB-CK activity confirmed the clinical diagnosis of infarction in 60 patients. In 53, the infarction was considered to be transmural and in seven it was nontransmural. Inferior transmural infarction was present in 20 and anterior infarction was diagnosed in 33. All except five of the 39 patients with transmural infarction who presented within 10 hr after the onset of symptoms had MB-CK values still within the normal range (n = 28) or serial MB-CK curves from which back-extrapolation confirmed onset of infarction within less than 10 hr from the time of sampling (n = 6). In three patients back-extrapolation indicated that the onset of infarction had been more than 10 hr before admission, but these three patients were retained in the “early” group for purposes of analysis. One patient had modestly elevated FPA levels (3.3 ng/ml); the other two had more marked elevations (8.38 and 35.4 ng/ml). Back-extrapolation was not possible with data from two other patients.

In 18 patients initially suspected infarction was not confirmed. In each the chest pain leading to hospitalization was thought to be of a cardiac cause. Results in one of these patients were excluded from the analysis prospectively because of difficulties associated with the sampling procedure.

FPA values. The mean FPA values from initial samples in patients with transmural infarction was 42.3 ± 11.2 ng/ml, significantly greater (p < .01) than that in patients with nontransmural infarction (4.8 ± 1.6 ng/ml) or in patients in whom the diagnosis of infarction was excluded (3.5 ± 0.6 ng/ml, table 2). No significant differences were noted between patients with anterior compared to those with inferior transmural infarction (33.2 ± 11.9 vs 56.2 ± 21.7 ng/ml).

All of the marked elevations in FPA level (>20 ng/ml) were noted in patients with transmural infarction from whom samples were obtained within 10 hr of
the onset of symptoms (figure 1). None of 13 patients in whom samples were obtained very early (less than 3 hr) had FPA values less than 8 ng/ml. In eight, elevation of levels of FPA was marked (>20 ng/ml). Patients with greater than 8 ng/ml FPA were admitted an average of 5.2 ± 0.8 hr after the onset of symptoms, i.e., significantly earlier than those with values less than 8 ng/ml (12.2 ± 2.0 hr, p < .001). When patients with transmural infarction were stratified into quartiles reflecting the interval from the onset of symptoms to the time of initial sampling, a progressive decline in mean FPA value with respect to time of onset of infarction was evident (figure 2).

In the retrospective analysis of values in patients with transmural infarction (figure 3), 10 hr after the onset of symptoms was identified as the boundary that best segregated samples from patients with high from those with low FPA. Mean FPA in patients from whom samples were obtained more than 10 hr after the onset

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (yr)</th>
<th>Gender (M/F)</th>
<th>CHF&lt;sup&gt;a&lt;/sup&gt; (n/%)</th>
<th>Severe arrhythmia&lt;sup&gt;b&lt;/sup&gt; (n/%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmural MI</td>
<td>53</td>
<td>60.1 ± 1.7</td>
<td>33/20</td>
<td>8/15</td>
<td>4/8</td>
</tr>
<tr>
<td>Anterior</td>
<td>33</td>
<td>57.6 ± 2.2</td>
<td>6/8</td>
<td>2/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Inferior</td>
<td>20</td>
<td>63.8 ± 2.3</td>
<td>4/8</td>
<td>1/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Samples obtained within 10 hr after onset of symptoms</td>
<td>39</td>
<td>60.5 ± 1.75</td>
<td>5/13</td>
<td>2/5</td>
<td></td>
</tr>
<tr>
<td>Samples obtained more than 10 hr after onset of symptoms</td>
<td>14</td>
<td>63.4 ± 3.5</td>
<td>3/21</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Nontransmural MI</td>
<td>7</td>
<td>53.1 ± 4.5</td>
<td>5/2</td>
<td>0</td>
<td>1/14</td>
</tr>
<tr>
<td>Infarction excluded</td>
<td>17</td>
<td>64.9 ± 2.8</td>
<td>12/5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>All patients</td>
<td>77</td>
<td>59.9 ± 1.3</td>
<td>50/27</td>
<td>9/12</td>
<td>5/6</td>
</tr>
</tbody>
</table>

MI = myocardial infarction; CHF = congestive heart failure.
<sup>a</sup>Class IV, according to the myocardial infarction research unit (MIRU) criteria.
<sup>b</sup>Ventricular tachycardia or fibrillation.
of symptoms was 55.5 ± 14.7 ng/ml compared with 4.9 ± 1.4 ng/ml in those sampled more than 10 hr after onset of symptoms (p < .01). Levels in samples obtained more than 10 hr after the onset of symptoms were not significantly different from those in samples from patients with nontransmural infarction or in samples from patients in whom acute infarction was excluded as a diagnosis.

Among the group of patients with transmural infarction in whom samples were obtained within 10 hr after onset of symptoms, only one patient had a normal level of FPA. Thirty of the 39 patients in this group (77%) had FPA values greater than 8 ng/ml. In contrast, among the patients in whom samples were obtained later than 10 hr after the onset of symptoms only, three had normal FPA values. Only two of the 14 patients in this group (14%) had FPA values of greater than 8 ng/ml. One had a mural thrombus present at the time of admission and the other had severe pericarditis.

Among patients in whom the suspected diagnosis of infarction was subsequently excluded, six of 17 patients had normal FPA values (<2 ng/ml). In only one (6%) was the level of FPA greater than 8 ng/ml.

Infarct size estimated in 33 of the 39 patients with transmural infarction and admitted within 10 hr after onset of symptoms averaged 23.9 ± 2.8 CK-geq. There was no correlation between infarct size and FPA level in the initial or subsequent samples (r = .16 for the initial sample, p > .05).

Ten patients underwent coronary angiography within 7 hr after the onset of symptoms for detection of thrombolysis. In each, total thrombotic occlusion was present. In nine, recanalization was induced by streptokinase or tissue-type plasminogen activator. In one, recanalization was not induced acutely but was present at repeat catheterization 10 days later. In each of these 10 patients levels were initially elevated (64.4 ± 26.6 ng/ml). Nine of the 10 had levels greater than 8 ng/ml.

Sequential changes in plasma FPA. Serial changes in plasma FPA were characterized in eight patients with transmural infarction from whom initial samples were available within 10 hr after the onset of symptoms. In each the FPA level was elevated in the initial sample. In seven of the eight, plasma FPA was greater than 8 ng/ml and it declined consistently throughout the 24 hr after admission (figure 4). In the eighth patient the initial value was elevated only modestly. Increased FPA persisted at approximately the same level over the first 24 hr.

In one patient with nontransmural infarction, the initial sample obtained within 10 hr after the onset of symptoms exhibited a normal FPA level (1.2 ng/ml). When this patient subsequently developed anterior transmural infarction, the FPA level in a sample obtained 30 min after the onset of the new symptoms was markedly elevated (13.25 ng/ml). The presence of coronary thrombosis was confirmed by angiography.

One patient with transmural infarction manifested early recurrent transmural infarction while FPA levels were being measured. FPA level was increased in the first sample after the onset of symptoms, auguring early recurrent infarction, and subsequently declined gradually (figure 5).

Fourteen patients with FPA values greater than 8 ng/ml were given heparin within the first 24 hr after the onset of infarction. In 11 of the 14 (79%) the FPA level decreased markedly (to a mean of a <15% of the original value) within 1 hr after the initial dose of heparin.
Discussion

FPA is a 16-amino acid peptide liberated as a product of thrombin-induced proteolysis of fibrinogen. Liberation of FPA and another 14-amino acid peptide, fibrinopeptide B, uncovers the E domain of fibrinogen. The residual protein, fibrin monomer, polymerizes to form a fibrin clot. Thus, liberation of approximately 4 ng/ml of FPA per milligram of fibrinogen is closely linked to clot formation. Because proteolysis of fibrinogen accelerates early after the onset of thrombosis, marked elevations in FPA levels are expected early after the onset of clotting.

Our results indicate that marked elevation in levels of FPA occurs early after the onset of transmural infarction. Values then decline consistently. The elevation of FPA levels is consistent with effects on fibrinogen of thrombin generated in association with the well-documented high incidence of early coronary thrombosis in patients with acute transmural infarction. Thus, the elevated FPA level appears to be a marker of coronary thrombosis in progress.

In other studies, only modest elevations of FPA level have been observed. Furthermore, the FPA magnitude did not appear to be a powerful descriptor of acute compared with chronic coronary disease. However, in most previous reports samples were generally not obtained early after the onset of symptoms. Thus, the mean values in these studies were comparable to those observed in patients in our study from whom samples were obtained more than 10 hr after the onset of symptoms. Some patients were evaluated earlier in the study of Mombelli et al., but these investigators included only a small number of patients who presented early after the onset of symptoms (mean duration of symptoms before sampling = 10 ± 9 hr) and did not consider separately patients with transmural, nontransmural, and those in whom localization of infarction was not possible. Nonetheless, four of 19 patients had FPA levels greater than 8 ng/ml and six had levels between 5 and 7 ng/ml. The elevated levels of FPA that may be anticipated early after the onset of infarction based on our results may not be evident if
patients hospitalized at variable intervals after onset of diverse types of infarction are considered without stratification. Because initially elevated FPA levels declined so promptly it is not surprising that the previous reports on patients sampled relatively late attested to only modest or inconsistent elevations.

The elevated FPA values we observed appear to reflect ongoing intravascular thrombosis rather than a nonspecific response to myocardial injury. Because the half-life of circulating FPA is 3 to 5 min, the amount of FPA liberated as a result of effects of thrombin in the intravascular compartment declines rapidly after administration of heparin, which inhibits activation of thrombin. Inhibition of activation of thrombin and reduction of circulating FPA occur more slowly when the locus is extravascular. The rapid decline in FPA we observed after administration of heparin suggests strongly that it was being generated in the intravascular compartment. If the source of activation of thrombin were reflective of injured myocardium itself, one would expect the magnitude of FPA elevations to correspond to enzymatically estimated infarct size. In fact, however, there was no relationship between infarct size and the extent of elevation of FPA level.

Although elevations in FPA level might have reflected formation of ventricular mural thrombus rather than coronary artery thrombus, ventricular thrombi were not generally detected even among the subset of patients evaluated echocardiographically because they were deemed to be at high risk. Furthermore, the time course of the elevation in FPA (early peak and prompt decline) is more consistent with an inciting coronary thrombus rather than a later evolving mural thrombus associated with myocardial ischemic injury. Thus, our data are more consistent with release of FPA from rapid fibrinogen turnover within an acute coronary thrombus.

The modest elevations in FPA level seen after more than 24 hr in patients with transmural infarction and those in patients with nontransmural infarction and with angina pectoris without infarction suggest that increased proteolysis of fibrinogen by thrombin may be associated with coronary artery disease per se. The marked initial elevations early after acute transmural infarction represent accelerated thrombosis with apparent pathogenic importance.

If such elevations in levels of FPA are indicative of acute coronary artery thrombosis, their detection may be particularly useful in selection of patients with infarction who have coronary arterial thrombi and are therefore particularly good candidates for treatment.
with thrombolytic agents. Markers of platelet degranulation such as platelet factor 4 and \( \beta \)-thromboglobulin unfortunately are not adequate for this purpose.\(^{21}\) Furthermore, elevation in FPA level may be a useful criterion for ongoing or recurrent clot formation and therefore may help to identify those patients at risk for complications of continuing or repeated coronary thrombosis.

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