Plasma Fibrinopeptide A Levels in Symptomatic Venous Thromboembolism

By I. M. Yudelman, H. L. Nossel, K. L. Kaplan, and J. Hirsh

Fibrinopeptide A (FPA) was measured in the plasma of 81 patients with suspected thromboembolism. Of 47 patients with positive venography and/or lung scan, 42 had elevated FPA levels >1.3 pmol/ml (mean 7.4) and 5 had levels <1.3 pmol/ml. Of 34 patients with negative venography and/or lung scan, 29 had FPA levels <1.3 pmol/ml and 5 had levels >1.3 pmol/ml. These results suggest limitations for the FPA assay as a sole diagnostic test for thromboembolism but indicate that the test is likely to be useful in symptomatic patients when used in addition to diagnostic methods such as venography, impedance plethysmography, and leg and lung scanning. Intravenous heparin (mean dose 100 U/kg) lowered the FPA level into the normal range within 15 min in 24 of 25 patients, indicating immediate suppression of thrombin action. Repeat lung scans, FPA levels, and activated partial thromboplastin times (APTT) were analyzed in 17 patients over the first 10 days of therapy. Three patients with significant new lung scan defects had elevated FPA levels on 5.2 days (mean) out of 10 and APTT <1.5 times the control value on 3 days out of 10. The other 14 patients with resolution or no change on the repeat lung scan had elevated FPA levels on 0.8 days out of 10 and APTT <1.5 times the control value on 1.1 days out of 10. The present findings relating clinical thrombosis to specific cleavage of the bond between residues 16 (Arginine) and 17 (Glycine) on the Aα chain of fibrinogen provide a basis for quantitatively linking specific biochemical changes in the hemostatic mechanism with thrombosis.

THE MAJOR AIM of heparin therapy in acute venous thromboembolism is to prevent further growth of the thrombus or embolus by inhibiting fibrin formation. Current recommendations are based on the results of experiments in animals1-3 and on the analysis of recurrent pulmonary embolism during treatment of patients.4 Since anticoagulants act by inhibiting the formation and action of thrombin, measurement in plasma of fibrinopeptide A (FPA), a specific product of thrombin action on fibrinogen, should provide direct information concerning the effectiveness of therapy in preventing fibrin formation in individual patients.

Plasma FPA levels were measured in patients with symptomatic venous thrombosis and pulmonary embolism with the aims of defining (a) FPA levels on presentation, (b) the effect of the initial injection of heparin on the FPA level, and (c) FPA levels during anticoagulant therapy and of relating these to clinical and laboratory evidence of recurrence.

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MATERIALS AND METHODS

Eighty-one patients (ages 24–82 yr, mean 56 yr) with symptoms suggesting venous thrombosis and/or pulmonary embolism were studied, 62 at the Columbia-Presbyterian Medical Center and 19 at McMaster University Medical Center. The clinical suspicion of thromboembolism was confirmed by venography or perfusion lung scanning in 47 patients, but in the other patients the venograms and lung scans were normal. Of the 47 patients with proven thromboembolism, 14 patients had within the previous month had tissue injury, including surgery (8 patients), fractured hip (2 patients), myocardial infarction (2 patients), and full-term delivery or abortion (2 patients). Four patients had pulmonary disease, including chronic obstructive pulmonary disease (two patients) or asthma (one patient). Four patients had neoplastic disease. Twenty-five patients did not have recent tissue injury or pulmonary or neoplastic disease. This group included one patient with angina, two patients who had had a long plane or automobile trip, and one who was obese. Of the 34 patients with a negative venogram and/or lung scan, 2 had neoplastic disease, and single patients had fractured ribs, acute pulmonary tuberculosis, or the nephrotic syndrome.

Diagnostic criteria for venous thromboembolism and for extension of the disease during therapy. Venous thrombosis was diagnosed by venographic demonstration of a constant filling defect that remained unchanged in shape and location and was seen in several projections in an otherwise well-opacified vein. Activity of the disease was diagnosed on clinical grounds. The diagnosis of pulmonary embolism was based primarily on perfusion lung scans with 99mTc albumin. Pulmonary embolism was diagnosed with high probability if a technically adequate scan with at least four views (anterior, posterior, right lateral, and left lateral) showed one or more well-defined lobar or segmental defects in regions that did not reveal corresponding abnormalities on a chest x-ray. 133Xe ventilation scans were performed in 2 patients with chronic obstructive pulmonary disease. Lung scans were repeated in 17 patients with pulmonary thromboembolism 7–10 days after the initial diagnosis both to confirm the diagnosis and to follow the patient’s response objectively. Repetition of the lung scans did not depend on the recurrence or persistence of symptoms.

Hematologic tests. Blood samples for coagulation tests were drawn before therapy at intervals after the first injection of heparin and thereafter daily. Samples for activated partial thromboplastin time (APTT) measurement were mixed with citrate (4.5 ml blood and 0.5 ml 3.8% trisodium citrate). The partial thromboplastin time was determined within 1 hr of blood collection. Samples for FPA measurement were mixed with heparin and trasylol [4.5 ml blood and 0.5 ml heparin (500 U) and trasylol (500 U)], processed, and assayed as previously described. Informed consent for drawing additional blood samples was obtained from each patient before the study was started.

Therapy. Treatment was initiated by an intravenous injection of heparin sodium (Upjohn, Kalamazoo, Mich.) 70–130 U/kg. Thereafter the heparin was administered as a continuous intravenous infusion, the rate of administration being adjusted with the aim of prolonging the partial thromboplastin time to 1.5–2 times the control value. Heparin was generally administered for 10 days. Sodium warfarin was generally started on the seventh day at 10 mg/day. Following cessation of the heparin infusion the warfarin dosage was adjusted with the aim of prolonging the prothrombin time to twice the control value.

RESULTS

Before heparin. In 86 normal individuals the mean FPA level was 0.6 pmol/ml, range 0.1–1.3 pmol/ml. In the 47 patients with confirmed thromboembolism the FPA level ranged from 0.4 to 112 pmol/ml, being <1.3 pmol/ml in 5 patients (Fig. 1). The mean level in the 47 patients was 7.4 pmol/ml and the median level was 3.4 pmol/ml. In the 34 patients in whom the venogram and/or lung scan was negative, the FPA level was normal in 29 and elevated in 5 patients (Fig. 1). Each of these 5 patients had acute tissue injury or neoplastic disease. FPA levels and associated conditions in the patients with confirmed thromboembolism are shown in Table 1. When the FPA levels were
analyzed in relation to the duration of symptoms, the levels were highest (9.7 pmol/ml) in patients with symptoms for less than 1 day, lower (4 pmol/ml) when symptoms had been present for 2–6 days, and least (2.8 pmol/ml) with more than 6 days of symptoms (Table 2).

Initial heparin infusion. The effect of the initial injection of heparin on the FPA level was studied in 25 patients. In 24 patients there was an immediate fall in the FPA level so that the normal range was reached in 10–30 min (Fig. 2). One patient showed no fall in FPA level after the initial heparin injection. In 3 patients FPA levels were measured 1–4 hr after the initial heparin injection and before the continuous heparin infusion was started; in 1 patient the FPA level returned to the initial level, and in the other 2 the level rose to 1.4 and 2.5 times the initial level between 1 and 2 hr after the heparin injection.

Table 1. Associated Conditions, Proven Thromboembolism

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of Patients</th>
<th>FPA Level (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>25</td>
<td>3.4</td>
</tr>
<tr>
<td>Tissue injury</td>
<td>14</td>
<td>3.3</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>4</td>
<td>3.7</td>
</tr>
<tr>
<td>Neoplastic disease</td>
<td>4</td>
<td>2.2</td>
</tr>
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</table>

Table 2. FPA Levels and Duration of Symptoms

<table>
<thead>
<tr>
<th>Duration of Symptoms (days)</th>
<th>No. of Patients</th>
<th>Mean</th>
<th>Median</th>
<th>Plasma FPA Level</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>9.7</td>
<td>9.0</td>
<td>0.4–20.3</td>
<td></td>
</tr>
<tr>
<td>2–3</td>
<td>8</td>
<td>4.0</td>
<td>3.3</td>
<td>0.7–12.0</td>
<td></td>
</tr>
<tr>
<td>4–5</td>
<td>11</td>
<td>4.1</td>
<td>2.7</td>
<td>1.0–10.6</td>
<td></td>
</tr>
<tr>
<td>6 or more</td>
<td>10</td>
<td>2.8</td>
<td>2.6</td>
<td>0.7–6.3</td>
<td></td>
</tr>
</tbody>
</table>
**Fig. 2.** Plasma FPA levels before and after the initial intravenous injection of heparin. Data points indicate mean FPA levels in 24 patients; SD, numbers next to data points. Zero point, time at which heparin was injected. Injection occurred over 30-sec period. Initial FPA level was determined on a blood sample drawn 1–2 min before heparin injection started; assumption was made that the FPA level had not changed in this 1–2 min period. Time periods on abscissa measured from start of heparin injection.

**FPA levels and clinical course during anticoagulant therapy.** FPA levels were measured daily in 21 patients, all of whom had positive lung scans and 17 of whom had a repeat lung scan. In 3 of these 17 patients, one of whom was free of symptoms during therapy, significant new defects in previously unaffected areas were present on the repeat lung scan, while in the other 14 patients the repeat lung scan revealed either complete or partial resolution (11 patients) or no change (3 patients). FPA levels and APTT results were analyzed on days 2–11 for each patient. In the 3 patients with evidence of new lung scan defects the FPA level was elevated a mean of 5.2 days out of 10, while in the 14 patients in whom there was resolution or no change the FPA level was elevated a mean of 0.8 days out of 10 (Table 3). The APTT was less than 1.5 times the control value on a mean of 3 days out of 10 in the 3 patients with new lung scan defects and a mean of 1.1 days out of 10 in the other 14 patients (Table 3).

The relationship between subjective evidence of recurrence and FPA levels was also examined. There was a close temporal association between recurrence

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Repeat Lung Scan</th>
<th>Elevated FPA, &gt; 1.3 pmol/ml (No. of Days out of 10)</th>
<th>Suboptimal PTT, &lt; 1.5 × Control (No. of Days out of 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>New defects</td>
<td>5.2 (5–6)</td>
<td>3 (1–6)</td>
</tr>
<tr>
<td>14</td>
<td>Resolved or unchanged</td>
<td>0.8*</td>
<td>1.1 (0–4)</td>
</tr>
</tbody>
</table>

*FPA levels were elevated on 11 days in total in these 14 patients. In one patient with venous thrombosis who had recurrent pain in the leg and in whom a repeat venogram was not done FPA levels were elevated on 6 days. In the other 13 patients FPA levels were elevated on 1 day only or not at all. The numbers in parentheses indicate the range in number of days (0–10) for which the number preceding the parentheses is the mean.
of the initial symptoms (chest or leg pain) and recurrent FPA elevations in 14 instances; in 9 instances recurrent symptoms were associated with normal FPA levels. The 3 patients who developed new defects on lung scan had four instances of recurrent symptoms associated with elevated FPA levels. Transient FPA elevations occurred in 10 of the 21 patients within 24-48 hr of stopping heparin, while the patients were receiving sodium warfarin. Of these 10 patients, 4 experienced a transient recurrence of symptoms.

DISCUSSION

The relationship between altered tests of hemostasis and thromboembolic disease has been the subject of a number of studies. With respect to pulmonary embolism and serum levels of fibrin/fibrinogen degradation products (FDP), Wilson et al. found elevated FDP in 24 of 25 patients and Rickman et al. in 18 of 19 patients, whereas Light and Bell found normal FDP in 29 of 35 patients. Our FDP data from an earlier study resemble those of Light and Bell. The differences in results may reflect differences in test technique or disease severity and/or acuteness of the disease in different studies. Positive statistical associations have been reported between thromboembolic disease and platelet function tests, altered fibrinogen behavior on gel chromatography, and other tests, including the protamine sulfate test for soluble fibrin. In asymptomatic venous thrombosis diagnosed by 125I-fibrinogen scanning, the correlation in individual patients with abnormal tests of hemostasis was too weak to be clinically useful.

In considering the FPA assay, the normal FPA level has been reported to be less than 1.3 pmol/ml, and elevated levels have been reported in sepsis, neoplastic disease, and systemic lupus erythematosus as well as with thromboembolism and disseminated intravascular coagulation. In the present study 42 of 47 symptomatic patients with documented thromboembolism had elevated FPA levels. The levels were generally highest in those patients who had symptoms for less than 1 day. Of the 47 patients, 25 had no antecedent disease or tissue injury. The median FPA level in this group was similar to that of the group as a whole, suggesting that the elevated FPA level was associated with the thrombosis rather than an underlying disease. Of 34 patients with a normal venogram and/or lung scan, 29 had normal FPA levels. All 5 of the patients with elevated levels had tissue injury or neoplastic disease.

These data indicate a close relationship between active fibrin formation, as reflected by elevated FPA levels, and thromboembolism. The data further indicate that although the FPA assay has limitations if used as a sole diagnostic test for thromboembolism, it is likely to be useful as clinical test in symptomatic patients when used in addition to other diagnostic methods. Practical implementation as a clinical test has been hampered by the cumbersome nature of the blood processing method, but a new technique, suggested by M. Blombäck, whereby the fibrinogen is adsorbed by bentonite and dialysis is avoided, appears to be satisfactory in our experience. Because it is essential that blood flow be free during collection, samples must be collected by trained personnel. The potential usefulness of assays for actively released platelet proteins as a supplement to the FPA assay needs to be explored.

The fall of FPA levels in 24 of 25 patients following the initial injection of
heparin confirms preliminary findings. The promptness of the decline at an initial rate consistent with its plasma half-life indicates virtually immediate neutralization in vivo of thrombin action at the dose of heparin used (mean 100 U/kg). In 2 patients FPA levels declined initially and then rose 1-2 hr after the bolus injection to levels higher than the initial level. This finding suggests that as the plasma heparin concentration declined, additional thrombin became available. There is insufficient data to relate the rise in FPA levels to the dose of the initial heparin injection. The reason for the failure of FPA levels to drop in 1 of the 25 patients is uncertain. Since the partial thromboplastin time was not measured after the initial heparin bolus, there is no objective evidence that the heparin entered the blood. This patient had normal antithrombin III levels by both functional and immunologic assay.

An unexpected result of this study was the association of elevated FPA levels with objective evidence on lung scanning of extension of the disease. FPA levels were elevated six times more frequently in the 3 patients with extension than in the other 14 patients whose repeat lung scans showed no extension. Of the 3 patients with new defects on lung scan, 2 were symptomatic during the first week of therapy, but the third patient was asymptomatic. The APTT was out of the prescribed range more frequently in the patients whose disease extended, but it is not clear whether extension of the disease resulted from suboptimal heparin therapy or whether extension increased the threshold of response to heparin therapy. The extension may have resulted from fresh leg vein thrombosis that embolized or from the breaking off and embolization of old thrombi associated with fibrin formation via accretion. The data available do not permit a distinction between these two possibilities.

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