Factors Affecting Fibrinopeptide-A Levels in Patients With Venous Thromboembolism During Anticoagulant Therapy

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The prompt reduction of elevated fibrinopeptide A (FPA) levels (normal < 1.3 pmole/ml) by heparin therapy in patients with thromboembolism suggests that measuring the FPA level may provide a good index of disease activity and be a useful method of monitoring therapy. Sepsis or malignancy may elevate FPA levels and coexist with thromboembolism. FPA levels were surveyed in 51 patients with thromboembolism (including 15 with concurrent sepsis or malignancy) during heparin treatment in an attempt to distinguish the effects of coexistent disease and the progression of thromboembolism. The anticoagulant effect of heparin was within the therapeutic range for 81% of the study period. In patients with thromboembolism alone and marked resolution of emboli on repeat lung scan, the mean daily FPA levels were lower than the values in patients with minimal resolution (p < 0.005). In patients with marked resolution of pulmonary embolism or venous thrombosis and a concurrent disorder, the mean FPA level remained elevated compared to normal values in patients with thromboembolism alone. These results suggest that FPA levels monitored during heparin therapy of thromboembolism may be useful as an index of disease activity except in the presence of coexisting sepsis or malignancy.

THERE ARE MANY factors influencing blood tests that reflect activation of hemostasis and hence influence the interpretation of such results.1 Fibrinopeptide A (FPA), a specific product of thrombin action in fibrinogen, can be measured in human plasma using a radioimmunoassay method.23 Recent clinical studies suggest that FPA levels reflect in vivo fibrin formation and may serve as an index of venous thromboembolism.45

The purpose of this project was to study plasma FPA levels during anticoagulant therapy in venous thromboembolism. Elevated FPA levels are not specific for thromboembolism and also occur in sepsis, malignancy, and immunologic disorders.6-9 Since venous thromboembolism and one of these other disorders may be present in the same patient, the effect of heparin in FPA levels in these situations may provide information about FPA generation in such patients.

Plasma FPA levels were measured before and during heparin therapy in a group of patients with venous thromboembolism alone and in a group with thromboembolism and a concurrent disorder.

MATERIALS AND METHODS

Patient Selection

Seventy-six patients with symptoms suggestive of deep vein thrombosis or pulmonary embolism were referred to the Hematology Service at the Brooklyn V.A. Medical Center over a 15-mo period. The patients were selected consecutively and on the basis of providing informed consent. In 51 patients (50 males and 1 female) aged between 33 and 80 yr, the presence of venous thromboembolism was confirmed.

Diagnostic Criteria

The presence of venous thrombosis was established by venography using the technique of Rabinov and Paulin.10 Pulmonary embolism was diagnosed by the presence of a filling defect on pulmonary angiography in 7 patients. In 29 patients, the appearance of multiple segmental or lobar perfusion defects seen in 2 or more views on a technically adequate perfusion lung scan, in the presence of a normal chest x-ray, suggested pulmonary embolism. Xenon-133 ventilation scans were performed in 15 of these patients, and the results supported the diagnosis of pulmonary embolism. In the remaining 14 patients with a high probability perfusion lung scan, ventilation scanning was not available and the presence of deep vein thrombosis on venogram was considered supporting evidence for pulmonary embolism. The perfusion defects were quantitated by expressing the area of perfusion defect on the scan as a percentage of the total perfusion to both lungs.11 The extent of resolution of pulmonary emboli was determined by repeating the lung scan after 8-10 days of heparin therapy. A decrease in size of the original perfusion defect by 50% or more represented significant resolution, whereas patients manifesting a smaller decrease were considered to have had minimal resolution.

The criterion of 50% reduction was adopted to allow clear-cut differentiation and was made prior to, and without knowledge of, the FPA results. The resolution of activity of deep vein thrombosis was determined clinically because noninvasive techniques were not available and because of the high incidence of discomfort following venography. The patients with malignancies all had radiologic and isotopic evidence of metastases. The diagnosis of sepsis was established by cultures of blood and secretions in 5 patients and was due mainly to gram-negative organisms.

Therapy

All the patients received porcine intestinal heparin (Panheparin, Abbott Laboratories, Chicago, Ill. or Liquaemin Sodium, Organon Laboratories, West Orange, N.J.) intravenously for 8-10 days either by injection every 4 hr or by constant infusion. The activated partial thromboplastin time (APTT) was performed once a day, and the heparin dose was adjusted to maintain the APTT between 1.5 and 2.5 times the control value. The APTT was determined by the

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Supported in part by funds from the Veterans Administration Medical Research Service.

Submitted May 20, 1981; accepted December 7, 1981.

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0006-4971/82/5904-0014$1.00/0

Blood, Vol. 59, No. 4 (April), 1982 787
automated clotting method using an automated APTT reagent (General Diagnosis, Morris Plains, N.J.) and citrated plasma (4.5 ml blood mixed with 0.5 ml 3.8% trisodium citrate). In patients receiving intermittent therapy, the APTT was performed between 30 and 60 min before the next dose. Warfarin sodium was started after 5 days of heparin therapy, and the heparin was discontinued when the prothrombin time approached twice the control value.

Hematologic Tests

Blood samples were obtained from patients immediately prior to therapy and then daily thereafter. All samples were carefully collected via a clean venepuncture with rapid flow of blood, immediately mixed with anticoagulant, and kept on ice until the plasma was separated (always less than 1 hr). Blood samples for FPA measurements were collected with a plastic syringe and mixed with heparin (1000 U) and Trasylol (1000 U) in a ratio of 1 volume anticoagulant to 9 volumes blood. 0.01M adenosine and 0.02M theophylline were subsequently added to the anticoagulant mixture so that the platelet factor 4 could be measured. These blood samples were collected directly into siliconized vacutainer tubes. The blood samples for FPA measurements were processed and assayed as previously described, except that the plasma was treated with bentonite instead of ethanol to remove the large fibrinogen molecules. Plasma samples for platelet factor 4 measurements were processed and assayed as described by Kaplan et al. Plasma antithrombin III levels were determined in citrated plasma by the amidolytic method using the synthetic chromogenic substrate S2238 (Ortho Diagnostics, Raritan, N.J.). The citrated plasma was prepared by centrifuging blood at 1700 g for 20 min at 4°C and then centrifuging the resultant plasma at 43,500 g for 10 min at 4°C. Serum fibrinogen degradation products (FDP) were measured by the tanned red cell hemagglutination inhibition immunoassay. Platelet counts were performed on alternate days with a Coulter Counter Model S (Coulter Electronics Co., Hialeah, Fla.)

Bleeding Complications During Heparin Therapy

In this study of 51 patients, there was one episode of bleeding. This occurred in a patient with documented gastric erosions whose APTT was prolonged beyond the therapeutic range. Platelet counts varied during heparin therapy, but no significant fall or count of less than 120,000/cu mm was observed.

Data Analysis

Statistical analysis was performed on test results obtained during periods of therapeutic anticoagulation. An analysis of variance was used to assess the statistical significance of the observed differences in the FPA levels (Fig. 1) and the antithrombin III, platelet factor 4, and fibrinogen degradation products concentrations between patients showing marked improvement and those showing minimal improvement (see Table 2). The Wilcoxon 2-sample test with continuity correction was used to evaluate the statistical significance of the observed changes in the FPA data in Fig. 2, since there was no homogeneity of variance. Variance increased significantly (p < 0.001) in patients with complications.
Results

Patients With Venous Thromboembolism Alone

The extent of the disorder and the predisposing conditions in 36 patients with venous thromboembolism alone are shown in Table 1. Cardiac failure was the cause of immobilization in 7 patients, and a neurologic abnormality was the cause in another 4 patients. The cardiac failure was due to coronary artery disease in 6 patients and an alcohol-related cardiomyopathy in 1 patient. The neurologic defects were hemiplegia due to recent cerebrovascular accidents in 2 patients, acute herniation of an intervertebral disc in 1 patient, and paraplegia due to a demyelinating process in 1 patient. Five patients had experienced an episode of venous thromboembolism within the preceding month but were not receiving anticoagulant therapy at the time of the study. Three patients had undergone surgery within the preceding 2 wk.

Plasma FPA Levels Prior to Heparin Therapy (Table 1)

Initial FPA levels were obtained in 26 patients, 10 with deep vein thrombosis and a normal lung scan and 16 with pulmonary embolism. All 10 patients with venous thrombosis had elevated FPA values, and the mean value was 3.4 pmole/ml (1.5–10.0 pmole/ml). Fifteen of the 16 patients with pulmonary embolism had elevated values, the mean value was 6.9 pmole/ml (1.0–14.0 pmole/ml). The highest values were found in patients with the shortest duration of symptoms.

Plasma FPA Levels and the Extent of Resolution of Pulmonary Embolism (Fig. 1)

The appearance of the repeat lung scan in patients with pulmonary embolism demonstrated that 7 patients had significant resolution of their original perfusion defects, and 17 patients had minimal resolution. The mean initial FPA value in these two groups was similar: 5.5 pmole/ml (1.0–14.0) in the minimal resolution group, and 4.5 pmole/ml (1.0–6.1) in the significant resolution group. FPA levels in all patients decreased during the subsequent period of heparin therapy. The mean daily FPA level in the group with minimal resolution remained elevated despite therapeutic levels of anticoagulant therapy. These values were significantly higher than the mean daily values in patients with marked resolution ($p < 0.005$) as shown in Fig. 1. After the twelfth day of treatment, the mean FPA level in the group with minimal resolution remained within the normal range for the duration of anticoagulation in hospital. In 2 patients with marked resolution of pulmonary embolism, but with radiologic evidence of small pleural effusions, the mean of the serial FPA values remained elevated: $3.6 \pm 1.0$ pmole/ml and $3.3 \pm 2.0$ pmole/ml. New perfusion defects were noted in 2 other patients; the mean FPA level was 2.5 pmole/ml in one and 0.95 pmole/ml in the other. Both patients exhibited marked resolution of the original defects and remained asymptomatic during the study period.

The clinical features of these two groups of patients are shown in Table 2. The age of the patients, duration of symptoms, incidence of cardiac failure and recent
venous thromboembolism, the size of the original embolism, and the mean daily heparin dose were similar in both groups. The APTT values were in the therapeutic range for 80% and 82% of the determinations in each group respectively. There was no significant difference between these 2 groups of patients with regard to abnormally low antithrombin III levels as well as mean FDP levels. Platelet factor 4 levels were measured serially in 5 patients in each group and were significantly higher in patients with marked resolution of pulmonary embolism ($p < 0.005$).

In all 10 patients with deep vein thrombosis alone, the clinical signs of the disorder subsided within 7 days. The mean FPA level during heparin therapy in these patients was 1.2 ± 0.4 pmole/ml.

Patients With Venous Thromboembolism and a Concurrent Disorder

Concurrent disorders consisted of: metastatic lung tumors, either squamous cell carcinoma (5 patients) or adenocarcinoma (2 patients); chronic lymphocytic leukemia (1 patient); polycythemia vera (1 patient); systemic lupus erythematosus (1 patient); and gram-negative sepsis (5 patients). The initial FPA level in 14 of these patients before heparin therapy and the extent of the thromboembolism is shown in Table 3. The mean FPA level of each group was markedly elevated, while the individual values ranged from mild elevations to the highest values found in the study. The effect of concurrent sepsis or neoplastic disorders on plasma FPA levels during therapeutic anticoagulation with heparin was analyzed in patients with marked resolution of pulmonary emboli and deep vein thrombosis (Fig. 2). In each category, patients with thromboembolism alone served as the control group. The mean FPA level in 10 patients with deep vein thrombosis alone was 1.2 ± 0.4 pmole/ml, which was significantly lower than the mean value of 3.8 ± 2.0 pmole/ml in 7 patients with concurrent disease ($p < 0.0001$). Similarly, the mean value in patients with pulmonary embolism alone was 0.9 ± 0.4 pmole/ml, which was significantly lower than the mean of 2.5 ± 1.8 pmole/ml in patients with a concurrent disorder ($p < 0.005$). The mean daily dose of heparin, required to achieve therapeutic anticoagulation, was similar in patients with venous thromboembolism alone and in those with coexisting disorders, 370(307–442) versus 320(200–445) U/kg/24 hr.

The effect of an underlying disorder on FPA levels was observed in two other situations during therapeutic levels of anticoagulation. One patient developed a urinary tract infection that responded to antibiotic therapy, and the patient with systemic lupus erythematosus had a temporary exacerbation of arthritis. FPA levels rose transiently from normal values to 3.0 pmole/ml in the former patient and to 2.4 pmole/ml in the latter patient.

Patients Without Venous Thromboembolism

Initial FPA levels were obtained in 13 of these 26 patients. Cellulitis of the legs or pneumonia was present in 9 patients, and the mean FPA level was 9.9 pmole/ml (1.6–22.0). In 4 patients, no acute disorder could be identified and the mean FPA level was 0.8 pmole/ml (0.3–1.2).

**DISCUSSION**

The results obtained in this study confirm the earlier observation that plasma FPA levels in patients with venous thrombosis and pulmonary embolism can be
used as a biochemical marker of intravascular fibrin formation.\(^4\) With regard to the sensitivity of FPA levels in this condition, 25 of 26 initial values were elevated in patients with thromboembolism and no other disorder (Table 1). Furthermore, the highest values were observed in those patients with the more acute form of thromboembolism as determined by the short duration of the symptoms. The effect of the extent of the thromboembolism on the initial FPA values was uncertain because the number of patients in each group was small.

The resolution of pulmonary embolism may be influenced by conditions such as preexisting cardiac disease, recurrent thromboembolism, the presence of reversible precipitating causes, and the size of the embolus, but the mechanisms responsible have not been identified with certainty.\(^17\)\(^22\) The persistence of elevated serial FPA levels coupled with minimal resolution of pulmonary emboli have both pathogenetic and therapeutic implications (Table 2). With regard to pathogenesis, persistently elevated FPA levels in these patients suggest ongoing intravascular fibrin formation that cannot be appreciated until the lung scan is repeated, despite the fact that 82% of the APTT determinations were in the therapeutic range. Persistent fibrin formation may be responsible for the impaired resolution. Pulmonary infarctions and pleural effusions, situations in which it has been suggested that FPA levels may remain elevated during heparin therapy, were not present here.\(^5\)

The anticoagulant effect as determined by the APTT values and the antithrombotic effect as reflected by elevated FPA levels were discrepant. The inhibition of thrombin by heparin depends primarily on the presence of antithrombin III, and low levels have been described in patients receiving heparin.\(^23\)\(^24\) Although there was a trend towards lower values in patients with minimal resolution, the sample size was too small for this difference to be considered statistically significant. Whether this is a major factor in preventing complete thrombin inhibition in these patients will be determined by correcting the antithrombin III deficiency.

Excessive heparin neutralizing activity in the plasma of patients with vascular disease has been attributed to the release of antitheparin material from platelets.\(^25\) While the exact influence of platelets on heparin in venous thromboembolism is unclear,\(^26\) the possibility that elevated platelet factor 4 levels could neutralize heparin was considered. However, the finding of normal values in patients where heparin's antithrombotic effect was impaired (elevated FPA levels) and elevated values in patients where heparin effectively inhibited thrombin action (normal FPA levels) does not support this speculation. The clinical sample was too small for accurate interpretation, and further studies are being carried out to define the role of platelet proteins in venous thromboembolism.

Elevated plasma FPA levels are not specific for venous thromboembolism and were observed in 9 patients with an acute disorder in whom thromboembolism was excluded. The appearance of fibrin in pathologic lesions of a diverse nature has been considered evidence of the activation of the blood coagulation system as part of the inflammatory response to these disorders. Using the plasma FPA level as an index of fibrinogen cleavage by thrombin, patients with systemic lupus erythematosus and a variety of solid tumors and leukemias have been investigated. The results of these studies suggest a close relationship between elevated FPA levels and evidence of disease activity. Conversely, during periods of remission, mildly elevated or normal FPA values were observed.\(^7\)\(^8\)\(^27\) A number of mechanisms by which activation of blood coagulation occurs in these disorders have been described. Leukocytes stimulated by bacterial endotoxin or immune complexes produce a thromboplastin-like material with lymphocytes amplifying the process.\(^28\)\(^31\) The release of tissue thromboplastin may occur as a result of a nonspecific event such as tissue necrosis. Tumor cells produce a procoagulant material as well as an enzyme that directly activates factor X.\(^32\)

It has also been proposed that elevated FPA levels in patients with malignancy may reflect subclinical disseminated intravascular coagulation. Attempts to distinguish intravascular from extravascular thrombin generation have been based on measuring the effect of heparin infusions on FPA levels and generation rates.\(^3\)\(^9\) The finding of persistently and transiently elevated FPA levels in anticoagulated patients with concurrent disorders in this study (Fig. 2) is comparable to the finding in cancer patients given heparin.\(^9\) Heparin has also failed to normalize elevated FPA values in patients with sepsis.\(^33\) These data strongly suggest that the fibrinogen cleavage is occurring at an extravascular site inaccessible to the anticoagulant. FPA produced in this manner could then leak into the blood where it can be measured.\(^34\) Alternatively, it is conceivable that the dose of heparin used in these patients was inadequate because of intense local intravascular procoagulant activity in sepsis or malignancy.

The finding of elevated FPA levels in patients with thromboembolism and the prompt reduction in these levels by heparin infusion suggests that measuring the FPA level may provide a good index of disease activity and be a useful method of monitoring therapy. Physiologically this would be logical because the FPA level is a sensitive index of the rate of fibrin formation in vivo. However, other diseases may elevate the FPA level and
coexist with thromboembolism. We therefore surveyed FPA levels in patients with thromboembolism during heparin treatment and attempted to distinguish the effects of coexistent disease and of progression of thromboembolism.

REFERENCES


ACKNOWLEDGMENT

We thank Dr. H. L. Nossel and Dr. K. L. Kaplan for their generosity in supplying us with isotopes and antisera for the FPA and platelet factor 4 radioimmunoassays.
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