Fibrinopeptide A Levels Indicative of Pulmonary Vascular Thrombosis in Patients With Primary Pulmonary Hypertension

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Although the mechanisms involved in the pathophysiology of primary pulmonary hypertension have not yet been delineated, thrombosis has been implicated. This study was designed to determine whether thrombin activity as reflected by plasma concentrations of fibrinopeptide A (FPA), a marker of the action of thrombin on fibrinogen, is increased in patients with primary pulmonary hypertension. To evaluate fibrinolytic activity, we measured plasma concentrations of tissue-type plasminogen activator, plasminogen activator inhibitor-1, and cross-linked fibrin degradation products. We studied 31 patients with primary pulmonary hypertension. Plasma FPA concentrations measured by radioimmunoassay, were elevated to 87.4±36.9 ng/ml (mean±SEM). Fifteen minutes after administration of heparin (5,000 U), FPA concentrations decreased to 6.8±1.4 ng/ml (p<0.001 compared with preheparin levels). In 21 of 30 patients (70%), FPA concentrations after heparin administration were less than half the preheparin levels, a response consistent with inhibition of thrombin by heparin and the short half-life of FPA. Despite evidence for marked thrombin activity, plasma concentrations of cross-linked fibrin degradation products were normal in all but four patients. Plasminogen activator inhibitor-1 activity was elevated in 19 of the 27 patients in whom it was measured, potentially limiting the fibrinolytic response. The elevations of FPA indicate that thrombin activity is increased in vivo in patients with primary pulmonary hypertension. Thus, sequential assays of plasma markers of thrombosis and fibrinolysis in vivo may help identify those patients who may benefit from treatment with anticoagulants. (Circulation 1990;82:841–847)

 Morphological examination of the pulmonary vasculature in patients with unexplained pulmonary hypertension reveals multiple small pulmonary artery "thromboemboli," plexogenic arteriopathy, or veno-occlusive disease.1,2 Although each of these morphological patterns may be associated with thrombosis, extensive, small pulmonary artery thromboemboli are the predominant feature in some patients. Because histological findings, as well as the lack of an obvious source for recurrent embolization, suggest that the small-vessel thromboemboli represent thrombosis in situ rather than embolization from extrapulmonary sources,2 this entity has been renamed thrombotic pulmonary arteriopathy. Although the mechanisms responsible for thrombosis in the pulmonary vasculature are not entirely clear, thrombosis may result from procoagulant stimuli, impaired inhibition of procoagulant activity, or a limited fibrinolytic response to intravascular thrombosis. Thrombosis may be associated with plexogenic lesions as well, perhaps in response to endothelial injury resulting from the elevated intravascular pressure. Anticoagulation has been reported to reduce mortality in patients with primary pulmonary hypertension, thus suggesting that inhibition of thrombosis in appropriately selected patients favorably alters the progression of the disorder.3

Concentrations of fibrinopeptide A (FPA), a small polypeptide released by the action of thrombin on fibrinogen, are elevated in plasma early after the onset of acute transmural myocardial infarction4 and in association with venous or arterial thrombosis.5-7 Because FPA is cleared rapidly from the circulation (half-life of 3–5 minutes), elevations of FPA that result from ongoing intravascular thrombin activity decline promptly in response to inhibition of throm-
bin when heparin is given. An impaired fibrinolytic response has also been observed in patients with thrombosis and may be an important determinant of the development of arterial thrombosis. This appears to be the case early after myocardial infarction when, despite increased thrombin activity, fibrinolysis is limited judging from the lack of elevations of cross-linked fibrin degradation products (XL-FDP) in plasma. Furthermore, survivors of myocardial infarction have increased concentrations of plasminogen activator inhibitor-1 (PAI-1) in plasma and decreased fibrinolytic activity in response to venous occlusion. A similar tendency toward a blunted fibrinolytic response has been demonstrated in patients who have had venous thrombosis.

The present study was designed to determine whether thrombin activity, as reflected by increased plasma concentrations of FPA, is increased in patients with primary pulmonary hypertension. Also, to determine whether fibrinolytic activity was decreased, we measured plasma concentrations of XL-FDP, tissue-type plasminogen activator (t-PA), and PAI-1. Patients were characterized prospectively with the use of clinical criteria and lung perfusion scans to determine whether specific subsets were associated with specific alterations in procoagulant or fibrinolytic activity.

Methods

Consecutive patients with well-documented primary pulmonary hypertension evaluated at the University of Illinois, Chicago, were studied. All vasoactive medications and oral anticoagulants were discontinued in study patients at least 2 weeks before blood samples were obtained. Initial clinical evaluation included a lung perfusion scan and right heart catheterization. Although pulmonary angiography was not performed as part of the evaluation, all patients met the National Institutes of Health registry guidelines for the diagnosis of primary pulmonary hypertension, and none had a ventilation-perfusion lung scan that indicated a high probability for pulmonary thromboembolism. None had a history of pulmonary thromboembolism or deep venous thrombosis. Results of noninvasive impedance plethysmography and Doppler echocardiography studies of the deep leg veins were normal in all patients. Right heart pressures were measured with fluid-filled catheters leveled to the midaxillary line and by standard methods. Blood samples for determining levels of FPA, XL-FDP, t-PA, and PAI-1 were obtained before further evaluation or treatment. Written, informed consent was obtained from all patients. The protocol was approved by the Human Study Committee at the University of Illinois and Washington University.

Blood Sampling Protocol

Blood samples for determining concentrations of FPA and XL-FDP were obtained by one of the investigators or a specially trained nurse at the time of cardiac catheterization in nearly all patients. In a few patients, initial samples were obtained by venipuncture. Initially, venous and, subsequently, arterial blood samples were obtained immediately after placement of the catheter-introducer sheath and before any further procedures were performed. The venous sample was obtained through an 8F sheath, and the arterial sample was obtained through a 5F Teflon cannula; these samples were obtained sequentially before any other indwelling catheters were placed. An initial 5-ml sample of blood was withdrawn and discarded before the sample was obtained for determining FPA concentration. Samples were then obtained 15 and 60 minutes after bolus administration of 5,000 U heparin i.v. A heparin and saline solution was used to flush the venous sheath between collections of each sample. Blood was drawn into precooled tubes containing EDTA, aprotinin, and d-phenylalanyl-l-prolyl-l-arginine chloromethyl ketone (PPACK), an inhibitor of thrombin. In addition, a sample for determining concentrations of PAI-1 and t-PA was drawn into a buffered citrate tube (Becton-Dickinson, Rutherford, N.J.) before administration of heparin. Samples were cooled rapidly to 4°C and centrifuged at 1,500g for 30 minutes to separate plasma. Plasma samples were stored at −70°C.

Fibrinopeptide A

For removal of fibrinogen, plasma samples were treated with bentonite adsorption before assay of FPA. FPA was measured by radioimmunoassay with antibody obtained from Mallinckrodt Inc. (St. Louis, Mo.). In our laboratory, the detectability of this assay is 1 ng/ml, and the upper limit of normal is 2 ng/ml. FPA concentrations were less than 1 ng/ml in plasma samples from four healthy, nonhospitalized volunteers; these samples underwent the same processing and shipping procedures as did those from the study patients. Intra-assay variability was 5.7±0.7%.

XL-FDP

Plasma concentrations of XL-FDP were determined by enzyme-linked immunosorbent assay (ELISA) with specific monoclonal antibodies from American Diagnostica, New York. A monoclonal antibody (DD3B6) specific for fibrin cross-linked between D regions (D-Dimer) was supplied coated on a 96-well microtiter plate. A 20-μl aliquot of plasma diluted at least 1:5 (vol/vol) was added to the microtiter plate and incubated for 1 hour. The plate was washed twice, and a second horseradish peroxidase–conjugated antibody specific for an epitope in the D region of fibrinogen was added. After a 1-hour incubation, the plate was again washed, and substrate [2-azino-di-(3-ethyl-benzthiazoline) sulphonate] (Kirkgaard Perry, Gaithersburg, Md.) was added. Absorbance was determined with an automated microtiter plate reader with the use of a commercial
analysis program (BioRad Laboratories, Richmond, Calif.). In our laboratory, the interassay variability for XL-FDP was 10.8±15.2% (n=55), but the interassay variability for samples above the upper limit of normal (> 300 ng/ml) was only 4.2±2.8%.

**Plasminogen Activator Inhibitor 1**

The fast inhibitor of t-PA present in plasma (PAI-1) was assayed with a modification of the spectrophotometric procedure developed by Chmielewska and Wiman. Predominantly single-chain melanoma t-PA was added to plasma samples to yield a final concentration of 10 IU/ml. The plasma was incubated for 10 minutes at room temperature, acidified, and “snap” frozen at −70°C to remove plasmin inhibitors. PAI-1 activity was determined by measuring the residual t-PA activity of samples with a chromogenic substrate for amidolytic activity of plasmin (S-2251, Kabi Vitrum/Helena, Beaumont, Tex.). One arbitrary unit (AU) of PAI-1 was defined as the amount that inhibited 1 IU t-PA under the conditions described, determined by comparing residual t-PA activity in the patient samples with a standard curve that was linear from 2 to 8 AU/ml. Assays in our laboratory yielded a mean PAI-1 level of 0.8±0.3 AU/ml (n=13) in samples from young nonhospitalized adults. The interassay coefficient of variation was 4.7±0.8%.

**Tissue-Type Plasminogen Activator**

Plasma concentrations of t-PA antigen were determined by enzyme-linked immunosorbent assay with antibody from American Diagnostica. In brief, a 96-well flat-bottomed microtiter plate (Immulon-2) was coated with purified goat antihuman t-PA immunoglobulin G. Plasma samples were acidified with 1.0 M sodium acetate, pH 3.9, for 15 minutes at 37°C and then neutralized. Purified human melanoma t-PA (final concentrations, 0.1–3.2 ng/ml) and plasma samples were applied to the plates and incubated for 3 hours at 37°C. The plates were washed, goat antihuman t-PA immunoglobulin G conjugated to horseradish peroxidase was added, and the samples were incubated at 37°C for 1.5 hours. Ortho-phenylene-diamine substrate with 0.1% H₂O₂ was added after the plates were washed. The reaction was terminated with 4.5 M H₂SO₄ after 30 minutes. Absorbance at 492 nm was determined with an automated microplate spectrophotometer (Titertek Multiskan, Flow Laboratories, Irvine, Calif.). The normal mean value for t-PA antigen of young nonhospitalized adults (in our laboratory) is 2.7±0.2 ng/ml (n=16).

**Analysis of Perfusion Lung Scans**

Perfusion lung scans were performed as part of the standard evaluation in all patients by the injection of 3 mCi of technetium 99m–labeled microaggregated albumin into a peripheral vein. Anterior, posterior, and oblique views were obtained with a scintillation camera and standard methods. Lung perfusion patterns in all patients were categorized by two of the investigators as normal (no perfusion defects), as indicative of patchy defects (with patchy distribution of uptake of tracer), or as indicative of intermediate defect (minimal, small perfusion abnormalities). In a previous study, it had been determined that patients with scans showing normal lung perfusion had plexogenic arteriopathy, whereas patients with patchy defects were more likely to have thrombotic pulmonary arteriopathy in lungs examined morphologically at autopsy.

**Statistical Analysis**

Data are presented as mean±SEM. Log transformation was used to normalize values for age, FPA, and XL-FDP. Data were compared with t tests (two tailed), χ² test with continuity correction, and Spearman’s correlation procedures.

**Results**

Blood samples were obtained from 31 patients diagnosed as having primary pulmonary hypertension (mean age, 37.2±2.0 years). Twenty-six were women. The mean pulmonary arterial pressure was 55.4±2.9 mm Hg (range, 33–100 mm Hg). Perfusion defects were patchy and diffuse in 14 patients. Lung perfusion scans were normal in only four patients, and scans showed intermediate perfusion defects in 13 patients. None of the patients was diagnosed as having pulmonary veno-occlusive disease based on chest radiograms, lung perfusion scans, and pulmonary arterial wedge pressures.

**Fibrinopeptide A**

Plasma concentrations of FPA were elevated in all patients; mean concentration was 87.4±36.9 ng/ml (normal, <2.0 ng/ml) (Figure 1). High elevations (>10.0 ng/ml) of FPA were observed in 61% (19 of 31) of the patients. Because of the unusually high elevations of FPA in the venous samples from some of the patients studied initially, an additional initial sample was drawn from the arterial catheter immediately after the sample had been obtained from the venous catheter in 16 patients. In 12 of these 16 patients, the duplicate samples confirmed that FPA was elevated markedly (>10 ng/ml) or modestly (2–10 ng/ml). In four of the 16 patients, FPA was elevated only modestly (2–10 ng/ml) in one sample and elevated markedly (>10 ng/ml) in the other sample, suggesting significant thrombin activity in vitro related to sample acquisition. For these 16 patients, the lower of the two FPA levels was used for analysis.

Baseline levels of FPA tended to be lower in the patients with scans showing normal lung perfusion (7.9±8.1 ng/ml) than in those with scans showing diffuse patchy perfusion defects or those with scans showing intermediate perfusion (20±68 and 80±198 ng/ml, respectively), but the differences were not significant. There was no relation between the elevation of FPA and duration of symptoms, age of the
patient, gender, or mean pulmonary arterial pressure.

Fifteen minutes after bolus administration of 5,000 U heparin, FPA concentration decreased to 6.8±1.4 ng/ml (p<0.001 compared with the preheparin level, n=30; one patient was not given heparin) (Figure 1). Sixty minutes after heparin administration, the mean FPA level had decreased further to 3.6±0.7 ng/ml. The FPA concentration decreased to half or less of the preheparin level within 15 minutes after heparin administration in 21 of 30 patients (70%) (Figure 2). Persistent elevations of FPA (>2.0 ng/ml) 15 minutes after heparin administration were observed in almost all patients in whom FPA concentration was markedly elevated initially; however, 60 minutes after heparin administration, FPA concentration decreased further. The patients in whom FPA levels did not decrease to half or less of the preheparin levels after heparin administration tended to have lower initial FPA levels; six of nine had levels less than 10 ng/ml compared with five of 21 patients who did have levels that responded to heparin (p=0.07) (Figure 3). FPA concentration did not decrease significantly in three of the patients with scans showing normal lung perfusion who were given heparin (one was not given heparin) compared with three of 13 patients with scans showing intermediate perfusion and three of 14 with scans showing patchy perfusion defects.

Markers of Fibrinolytic Activity

Despite marked thrombin activity reflected by FPA elevations, plasma concentrations of XL-FDP were normal in all but four patients (mean, 121±26 ng/ml; normal, <300 ng/ml; Figure 4). In three of the four patients, elevations were minimal (300–400 ng/ml). Each of the four patients with elevated levels of XL-FDP had markedly elevated levels of FPA (857, 142, 32.5, and 19.9 ng/ml).

PAI-1 activity in plasma was elevated in 19 of 27 patients in whom it was measured (4.3±0.8 AU/ml, n=27; Figure 4). The plasma concentration of t-PA from these patients was 13.2±4.4 ng/ml (n=27). There was no association between the extent of elevation of PAI-1 or XL-FDP level and the assignment of patients to subsets based on analysis of lung perfusion scans.

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

*Figure 1.* Bar graph of fibronopeptide A (FPA) concentrations in patients with primary pulmonary hypertension. Baseline FPA concentration was markedly elevated and decreased 15 minutes after a bolus administration of heparin (5,000 U i.v.), which is consistent with the short half-life of FPA in plasma (3–5 minutes) and with the inhibition of intravascular thrombin. FPA levels after heparin administration were significantly lower than those at baseline (p<0.001, paired t test).

![Figure 2](http://circ.ahajournals.org/)

*Figure 2.* Plot of fibronopeptide A (FPA) concentrations in patients in whom FPA levels decreased by at least 50% within 15 minutes after heparin administration. Although FPA concentration decreased significantly, levels remained elevated in some patients, particularly those in whom FPA levels had been markedly elevated at baseline.
Discussion

The elevated levels of FPA we observed indicate that thrombin activity is increased in most patients with primary pulmonary hypertension. The prompt decrease of FPA levels after heparin administration is consistent with intravascular thrombin activity as the cause of the initial increases. Despite having increased thrombin activity, the patients of this study did not manifest elevated levels of XL-FDP, which is a marker of fibrinolytic activity, and activity of PAI-1 in plasma was increased. Our results are consistent with histological findings by other investigators and indicate that pulmonary vascular thrombosis is associated with primary pulmonary hypertension, perhaps as a primary mechanism in thrombotic pulmonary arteriopathy or as a secondary phenomenon in some patients with plexogenic arteriopathy.2

Intravascular thrombosis is often present in the small pulmonary arteries of patients with primary pulmonary hypertension. Because intact vascular endothelium inhibits platelet aggregation and thrombosis, the occurrence of small-vessel thrombosis is consistent with the view that endothelial injury is a primary event in some patients with primary pulmonary hypertension and results in increased pulmonary vascular resistance and pressure, which, in turn, may promote further endothelial damage. Structural changes in the pulmonary vascular endothelium have been observed with both primary and secondary pulmonary hypertension and have been associated with increased concentrations of factor VIII as well as abnormalities in the structure of circulating von Willebrand factor polymers, the latter perhaps reflective of abnormal synthesis by endothelial cells.30,31 Increased plasma concentrations of FPA indicate the presence of a procoagulant stimulus that results in thrombin activity and fibrin formation, which in our patients may or may not be localized to the pulmonary circulation.

In patients with primary pulmonary hypertension, thrombosis may be exacerbated by inadequate fibrinolytic activity. Increased PAI-1 activity is associated
with an inadequate fibrinolytic response and is consistent with a prolonged euglobulin lysis time in patients with primary pulmonary hypertension. Increased PAI-1 activity has been found in patients predisposed to other thrombotic disorders, such as myocardial infarction and deep venous thrombosis. Although the plasma concentration of t-PA was not decreased, in the face of increased PAI-1 activity, the t-PA level in the pulmonary vasculature may be insufficient to induce fibrinolysis.

Despite meticulous sample acquisition techniques, we found that very high elevations of FPA (>100 ng/ml) in a few patients could be attributed in part to thrombin activity in vitro generated at the time of sample acquisition. Although our results require a cautious interpretation of the absolute magnitude of the observed FPA elevations, the confirmation of high elevations of FPA in multiple samples from multiple sites in all but four patients and the persistence of increased FPA levels after administration of heparin only in patients in whom FPA was initially increased suggest that the increases in FPA observed are due to a significant increase in thrombin activity in vivo in patients with primary pulmonary hypertension.

In a few patients, FPA concentrations did not decrease in response to heparin. This may reflect the release of FPA from an extravascular site of thrombin activity that is not rapidly inhibited by intravenous heparin, failure of heparin–antithrombin III to inhibit intravascular thrombin activity, or crossreactivity of the assay FPA with elastase-derived fibrinopeptide Aa 1-21, which may be elevated in patients with chronic obstructive pulmonary disease. We also found that patients with scans showing normal lung perfusion tended to have lower initial levels of FPA that did not decrease after heparin administration, but this difference was not significant, perhaps because of the small number of patients with normal lung perfusion. The lower levels of FPA in these patients is consistent with the findings of previous studies in which patients with scans showing normal lung perfusion were found to have plexogenic arteriopathy, a histological pattern in which thrombosis may be present but is not a prominent feature. However, in the present study, levels of FPA were increased in many of the patients with isolated small perfusion defects (intermediate defects), a group that would have been categorized as having normal lung perfusion based on previous criteria. This is not surprising because a biochemical marker of thrombin activity should be more sensitive than criteria based on radionuclide perfusion scanning. Additional prospective studies will be necessary to determine whether an increased FPA level is a specific marker of pulmonary vascular thrombosis in patients with primary pulmonary hypertension.

Our results do not distinguish increased thrombin activity due to endothelial injury as a result of increased pulmonary vascular pressures from increased activity due to a primary procoagulant stimulus. In addition, we cannot be certain that the site of thrombin activity is the pulmonary vasculature. To localize thrombin activity, it will be necessary to document an increase in FPA concentration in the aorta compared with that in the pulmonary artery. Unfortunately, acquisition of samples through indwelling catheters often results in activation of the coagulation system and increases in the FPA concentration due to in vitro thrombin activity. Even with a meticulous sampling technique, we found that some of the increase in FPA may have been attributable to the fact that samples were obtained through venous and arterial catheter sheaths. Improved methods for bonding of heparin to catheters may decrease their thrombogenicity and allow for acquisition of samples from the pulmonary artery in future studies.

Because thrombosis is not present consistently and because of the variability in the intensity of thrombin activity observed in our study, it is likely that only selected patients will benefit from interventions designed to inhibit thrombosis or enhance fibrinolysis. Measurement of FPA, XL-FDP, and PAI-1 activities should permit practical, rapid, and noninvasive characterization of procoagulant and fibrinolytic activity in individual patients over time. Elevation of FPA and a prompt decrease in response to heparin may identify a group of patients in whom thrombosis is likely to play an important role in the pathogenesis of pulmonary hypertension, patients in whom anticoagulation may be beneficial.

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