Evidence for Reduced Fibrinolytic Activity in Unstable Angina at Rest
Clinical, Biochemical, and Angiographic Correlates

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Background. The goal of this study was to evaluate the role of the fibrinolytic system in patients with unstable angina at rest associated with transient electrocardiographic changes.

Methods and Results. Tissue plasminogen activator activity in plasma was comparable among patients with unstable angina (n=17), patients with stable exertional angina (n=10), and control patients with normal coronary arteriograms (n=8). In contrast, plasminogen activator inhibitor-1 (PAI-1) activity in plasma was elevated in the unstable angina group (21.67±9.52 AU/ml) as compared with either the stable angina group (12.01±7.06 AU/ml, p<0.02) or the controls (12.49±8.54 AU/ml, p<0.02). Coronary angiography performed within 24 hours after the last anginal episode showed a similar extent of coronary artery disease in the unstable and stable angina groups. However, intracoronary thrombi were observed in eight patients in the unstable angina group while no thrombus was noted in the stable angina group (χ²=7.22, p<0.01).

Conclusions. We conclude that patients with unstable angina at rest have a reduced fibrinolytic activity and an increased incidence of intracoronary thrombi. It is postulated that elevated PAI-1 activity in the presence of coronary arterial wall injury may be an important factor leading to the development of acute coronary syndromes. (Circulation 1991;83:1685–1691)

Vessel wall injury and intracoronary thrombosis are salient features of the acute coronary syndromes such as unstable angina and myocardial infarction. The pathogenesis of unstable angina is especially difficult to ascertain since this disorder comprises a heterogeneous patient population (e.g., those with new-onset angina, postinfarction angina, or crescendo effort and rest angina). Furthermore, the extent of intravascular thrombosis in patients with unstable angina is likely to be smaller than in those with transmural myocardial infarction. Platelet aggregation and clotting system activation have been implicated in the process of intracoronary thrombosis in patients with unstable angina, but abnormalities in the fibrinolytic system have received less attention. Plasminogen activation plays a pivotal role in intravascular fibrinolysis, preventing the accumulation of fibrin. The central components of the fibrinolytic system consist of tissue plasminogen activator (t-PA), produced mainly by endothelial cells, and plasminogen activator inhibitor-1 (PAI-1), circulating in the blood and present in endothelial cells, platelets, and the liver. The affinity of endogenous t-PA for fibrin leads to plasmin generation in the vicinity of thrombus, which then potentiates local fibrinolysis.

Accordingly, the goal of this study was to evaluate these two components of the fibrinolytic system in patients with unstable angina at rest and angiographically documented coronary artery disease as compared with patients with either stable exertional angina or atypical chest pain.

Methods

Patient Population

The study population consisted of 35 patients admitted to Thomas Jefferson University Hospital for the evaluation of chest pain. These patients were divided into three groups: those with unstable angina, those with stable exertional angina, and the controls. Unstable angina (n=17) was defined as resting chest pain with documented transient ST segment depression, ST segment elevation of at least 0.1 mV, or T wave inversion in at least two contigu-
ous electrocardiographic (ECG) leads. The last episode of chest pain was required to have occurred within 12 hours prior to entry into the study. Patients with acute myocardial infarction, defined as new Q wave development or an increase in the creatine kinase concentration to more than twice the normal upper limit during the 24 hours after entry into the study, were excluded. The creatine kinase concentration was measured every 4 hours for 24 hours following enrollment. Stable angina (n=10) was defined as typical exertional chest discomfort, with the most recent episode occurring 2 or more days prior to study entry. No change in the frequency of angina, precipitating factors, or response to sublingual nitroglycerin was noted for the past 3 months in this study group. These patients were admitted for elective cardiac catheterization. The control group (n=8) consisted of patients with atypical chest pain and normal coronary arteriograms.

No patient had recent surgery, trauma (within past 3 months), known malignancy, or infection or had received oral anticoagulant or steroid therapy.

**Study Protocol**

All patients underwent venous blood sampling after admission. To account for circadian variations in plasma t-PA and PAI-1 levels, the time of the admission blood sampling was matched (+2 hours) for patients in the unstable and stable angina groups. Patients with unstable angina underwent diagnostic cardiac catheterization within 24 hours after the last episode of chest pain. In 15 of the 17 patients in the unstable angina group, repeated measurements of fibrinolytic activity prior to hospital discharge were obtained. The time of predischARGE blood sampling in individual patients was comparable to the time of admission blood sampling (+2 hours). In the remaining two patients, predischARGE blood samples were not available (one patient died and the other patient refused venipuncture).

**Blood Sampling**

Venous blood samples were obtained in the resting state without the use of a tourniquet. In addition, t-PA antigen release was evaluated after veno-occlusion, which was produced by a blood pressure cuff applied to the upper arm at 100 mm Hg for 10 minutes. The first 3 ml of blood was discarded; then 1.8 ml of venous blood and 0.2 ml sodium citrate (0.13 mol/l, pH 7.5) were mixed, placed on melting ice, and centrifuged at 2,000g for 10 minutes at 4°C. For measurements of t-PA activity, the citrated plasma samples were immediately acidified with sodium acetate buffer (1.0 M, pH 3.9). The acidified and citrated plasma samples were stored at 70°C until analyzed.

**Determination of Plasma t-PA and PAI-1 Antigen Concentrations**

Enzyme-linked immunosorbent assay (ELISA) with reagents supplied by American Diagnostica Inc., Greenwich, Conn., was used to determine the concentration of t-PA antigen in plasma. PAI-1 antigen in plasma was measured with a PAI-1 ELISA kit (Zymogen, Charlottenlund, Denmark). The absorbance at 490 nm was determined with an eight-channel scanning spectrophotometer.

**Determination of Plasma t-PA and PAI-1 Activities**

Activity of t-PA was measured in acidified plasma samples by means of a chromogenic assay. The samples were diluted 40-fold with 0.05 mol/l Tris-HCl buffer, pH 8.8, containing 0.1 mol/l NaCl and 0.1 g/l Triton X-100. An equal volume of reaction mixture consisting of final concentrations of 0.1 mg/ml plasminogen, 0.6 nmol/l S-2251, and 0.25 mg/ml poly-l-lysine was added. The samples were subsequently incubated at 37°C for 2 hours, and the absorbance at 405 nm was recorded. The t-PA activity used as a standard was that of double-chain t-PA (provided by Henry Berger, PhD, Burroughs Wellcome Co., Research Triangle Park, N.C.), which was added to acidified pooled plasma to yield the standard curves. The lower limit of sensitivity of this assay is 0.01 IU/ml.

The PAI-1 concentration was quantified in plasma samples with a modification of a chromogenic assay. Standard t-PA was added to plasma samples in a final concentration of 2 IU/ml, followed by acidification (1.0 M sodium acetate buffer, pH 3.9). An aliquot of the same plasma sample without added t-PA was acidified to determine the background t-PA activity. Residual t-PA activity and background t-PA activity were assayed with the same reaction mixture as described above. Standard curves were prepared concomitantly in acidified pooled plasma from young healthy donors. PAI-1 activity in each sample, corrected for its corresponding plasma background, was determined with respect to standard curves and expressed in arbitrary units (AU) per milliliter. One AU of PAI-1 is defined as the amount that inhibits 1 IU of t-PA activity over 15 minutes. The lower limit of sensitivity of this assay is 0.01 IU/ml.

For the above assays, the intra-assay coefficient of variation was below 5% and the interassay coefficient of variation was below 10%.

In addition, serum levels of total cholesterol and triglycerides in the fasting state were measured by standard enzymatic methods.

**Coronary Angiography**

Cardiac catheterization was performed in 34 patients (one patient from the unstable angina group refused cardiac catheterization). Selective coronary angiography was performed in multiple orthogonal views. Coronary angiograms were reviewed independently by three experienced angiographers without knowledge of the clinical presentation or results of biochemical analyses. The angiographic parameters analyzed included the number of vessels with luminal diameter stenosis of at least 70% and the presence of coronary collaterals.
In addition, the “angina-producing” coronary artery was identified based on the location of resting ECG changes (in the unstable angina group) or the results of an exercise stress test (in the stable angina group). Stenosis in the angina-producing coronary artery was classified as either simple or complex using a modification of the previously described methodology. Simple lesions were those having a smooth, tapered configuration without intraluminal lucencies. Complex lesions were those having irregular, hazy, or ulcerated borders or overhanging edges. Intracoronary thrombus was defined during diastole as either contrast media staining or a filling defect in the angina-producing coronary artery.

Statistical Analysis

All results are expressed as mean±SD. The data were analyzed by Kruskal-Wallis one-way analysis of variance; subsequent comparisons between groups were carried out with Wilcoxon’s rank sum test for unpaired data. Discontinuous variables were assessed using Fisher’s exact test with Bonferroni’s correction where appropriate.

Patient Characteristics

Patient baseline characteristics were similar among the study groups (Table 1). Women were more prevalent in the unstable angina group (11 of 17) than in the stable angina group (two of 10) and the control group (two of eight). As expected, patients with unstable angina were treated more frequently with antianginal medications, heparin, and aspirin than those with stable angina. No patient was receiving intravenous heparin at the time of predischarge blood sampling.

Assessment of Plasma t-PA and PAI-1

Baseline t-PA activity (Figure 1) was 8.29±4.22, 7.79±3.31, and 8.56±2.89 IU/ml in the unstable angina, stable angina, and control groups, respectively (differences not significant). There were no significant differences in the baseline t-PA antigen concentrations among the three study groups (Table 2). The t-PA antigen concentrations after venooclusion were also comparable among the study groups, increasing on the average approximately two-fold. In the 15 patients from the unstable angina...
group who had predischarge measurements (ranging from 2 to 33 days after admission), no significant differences in t-PA activity or t-PA antigen concentration at baseline and after veno-occlusion were noted between the admission and predischarge samples (Table 3).

Baseline PAI-1 activity in plasma differed significantly among the study groups (Figure 1). It was 21.67±9.52 AU/ml in the unstable angina group, 12.01±7.06 AU/ml in the stable angina group (p<0.02 versus unstable angina group), and 12.49±8.54 AU/ml in the controls (p<0.02 versus unstable angina group and not significant versus stable angina group). In the unstable angina group, plasma PAI-1 activity was not affected by the interval from chest pain to sampling or the direction of ST segment shift on the surface ECG during pain; no correlation was found between PAI-1 activity and serum triglycerides (y=17.46+0.02x, r=0.27). In addition, therapy with intravenous heparin at the time of enrollment had no significant effect on PAI-1 activity (22.10±10.80 AU/ml in eight patients on heparin and 21.18±8.56 AU/ml in nine patients not treated with heparin). In the unstable angina group PAI-1 activity remained elevated at the time of hospital discharge, when the patients were considered clinically stable and no intravenous medications (including heparin and nitroglycerin) were administered (Table 3). There was no significant correlation between PAI-1 and t-PA activities in the three study groups (y=21.9−0.6x, r=0.23).

**Coronary Morphology**

Results of coronary angiography in the unstable and stable angina groups are presented in Table 4. The extent of coronary artery disease and collaterals to the angina-producing vessel was similar in the two groups. Furthermore, the severity and morphology of coronary lesions in the angina-producing vessel were comparable between the groups. However, intracoronary thrombus was noted in eight patients from the unstable angina group, while no thrombus was found in the stable angina group (x²=7.22, p<0.01). The t-PA and PAI-1 activities were similar in patients with and without intracoronary thrombi in the unstable angina group.

**Discussion**

This study demonstrated reduced fibrinolytic potential and increased intracoronary thrombosis in the patients with unstable angina pectoris compared with patients with either stable exertional angina or atypical chest pain and normal coronary anatomy.

**Pathophysiology of Unstable Angina**

There is ample evidence that transient reductions in coronary blood flow precipitate episodes of resting angina. These changes are related to vessel wall injury and often associated with abnormal vasomotor reactivity. Coronary angiographic anatomy in patients with unstable angina is marked by the presence of significant coronary lesions and frequent intraluminal thrombi, which distinguish these patients from those with stable angina. This study demonstrated...
intracoronary thrombus in 50% of the patients with unstable angina at rest, which is similar to the findings of Gotth et al. The true incidence of intracoronary thrombi in these patients, however, is most likely higher, as suggested by angiographic findings.

Several abnormal hemostatic mechanisms have been described in patients with unstable angina. Endothelial denudation and increased shear forces related to vessel wall injury result in platelet activation and aggregation. This has been associated with an increased release of thromboxane A2, and other platelet-derived factors such as platelet factor 4 and β-thromboglobulin. Platelet involvement in the pathogenesis of unstable angina was also corroborated by large clinical trials with aspirin, an inhibitor of thromboxane A2 formation. Although this treatment reduced mortality and the incidence of myocardial infarction, some patients remain refractory to medical therapy. This may be explained in part by the presence of the severe coronary artery stenosis associated with platelet and clotting system activation unrelated to the arachidonic acid pathway. Accordingly, Theroux et al have demonstrated high levels of fibrinopeptide A in patients with unstable angina, which presumably reflects increased intracoronary thrombosis. Furthermore, Kruskal et al found increased concentrations of D dimer and other fibrin-related antigens in patients studied within an hour after anginal pain. The elevated D dimer concentration in patients with unstable angina at rest is most likely due to enhanced formation of cross-linked fibrin clot, to intracoronary thrombosis, and to the continuous breakdown of cross-linked fibrin.

**Fibrinolytic System in Coronary Artery Disease**

Conflicting data exist regarding fibrinolytic activity in patients with stable coronary artery disease. Our data indicate comparable resting levels of plasma t-PA and PAI-1 in patients with stable exertional angina and control subjects. It is possible, however, that impaired fibrinolytic activity exists in patients with stable angina, as evidenced by reduced t-PA release following exercise as reported by others.

This study demonstrated a significant elevation of plasma PAI-1 activity in patients with unstable angina at rest. There are several possible explanations of these findings. First, vessel wall injury in patients with unstable angina may result in the release of PAI-1 and growth factors (e.g., transforming growth factor) from activated platelets. The latter could stimulate synthesis of PAI-1, as evidenced by an increase in PAI-1 messenger ribonucleic acid hepatoxy cell cultures after incubation with platelet lysates. This mechanism, however, is unlikely since the PAI-1 activity was elevated in our study without an increase in the PAI-1 antigen levels. Second, transformation of the PAI-1 molecule from its latent to its active form could explain the elevation of plasma PAI-1 activity without an increase in the PAI-1 antigen concentration in the unstable angina group. The effects of plaque rupture and the exposure of matrix components in the vessel wall on regulation of PAI-1 activity remain unknown. However, phospholipids, the major components of cellular membranes, have been shown to activate latent PAI-1 in vitro. In addition, it has been demonstrated in vitro that bidirectional changes between latent PAI-1 and active PAI-1 could occur. Third, PAI-1 is an acute-phase reactant, and its plasma activity increases briefly in response to several nonspecific stimuli. It is unlikely, however, that the observed higher PAI-1 activity in patients with unstable angina was related to an acute-phase response since the three study groups were comprised of hospital patients who underwent the same invasive procedure (i.e., cardiac catheterization). Finally, hypertriglyceridemia has been associated with an elevated plasma PAI-1 activity; however, serum triglycerides did not differ significantly among the three study groups in the present study.

There are certain limitations to our findings. Specifically, the limitations are related to the small number of observations and the possibility that the elevated PAI-1 activity in the unstable angina group reflected an increase in other plasminogen activator inhibitors since the PAI-1 antigen concentration was comparable among the groups.

**Clinical and Therapeutic Implications**

The major function of PAI-1 is to form inactive complexes with circulating t-PA. Hence, an excess of plasma PAI-1 may result in impaired fibrinolytic activity in the vicinity of a ruptured atherosclerotic plaque, as reflected by prolonged clot lysis in the presence of a high plasma PAI-1 activity. Thus, it is postulated that the elevated PAI-1 activity in patients with unstable angina at rest may be an important factor leading to abnormal intracoronary hemostasis, which is clinically manifested as acute coronary syndromes. An increased plasma PAI-1 activity has also been reported in patients with acute myocardial infarction. It has been postulated that following thrombolytic therapy in patients with acute myocardial infarction, coronary artery reocclusion is promoted by reduced fibrinolytic activity in addition to other recognized factors such as the severity of residual stenosis and platelet activation. Higher plasma PAI-1 activity has been found in patients with occluded infarct-related arteries 72 hours following reperfusion therapy. Interestingly, an elevated PAI-1 activity persists in some patients after transmural myocardial infarction during long-term follow-up. The adverse prognostic role of an elevated PAI-1 activity has been suggested by an increased risk of reinfarction in those patients during the next 3 years.

Conventional therapeutic modalities seemed to exert minimal, if any, effects on PAI-1 activity in patients with unstable angina in the present study. None of these therapies, however, alleviates vessel wall injury, which is likely responsible for the ob-
served abnormalities. Although clinical quiescence at the time of hospital discharge could be achieved as a result of coronary revascularization, persistent vessel wall injury was likely responsible for the continued elevation of PAI-1 activity. Changes in PAI-1 activity following bouts of unstable angina during long-term follow-up remain to be determined. The elevated plasma PAI-1 activity associated with a high incidence of intracoronary thrombus found in this study could provide the rationale for thrombolytic therapy to enhance local fibrinolysis in selected patients with unstable angina at rest. Furthermore, recent advances in molecular biology indicate that the expression of t-PA and PAI-1 genes are regulated independently. Accordingly, a selective increase in t-PA synthesis and secretion into the conditioned medium of endothelial cell cultures has been shown to occur in response to pharmacologic stimulation with sulfonylureas or polyamines. If these cell culture studies can be reproduced in vivo, this may result in the development of novel pharmacologic therapies augmenting endogenous fibrinolysis.

In conclusion, the present study showed that unstable angina at rest was associated with an elevated PAI-1 activity that persisted during short-term follow-up despite conventional therapy. Abnormal fibrinolytic activity in unstable angina was accompanied by a high incidence of intracoronary thrombi, which suggest that increased PAI-1 activity plays an important role in the development of acute ischemic syndromes.

Acknowledgment

We gratefully acknowledge the expert secretarial assistance of Ms. Laraine Bartlett.

References


**Key Words** • tissue plasminogen activator • plasminogen activator inhibitor-1 • intracoronary thrombi • unstable angina
Evidence for reduced fibrinolytic activity in unstable angina at rest. Clinical, biochemical, and angiographic correlates.
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Circulation. 1991;83:1685-1691
doi: 10.1161/01.CIR.83.5.1685

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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