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# Fibrin Metabolism in Patients with Acute Myocardial Infarction During and After Treatment with Tissue-Type Plasminogen Activator

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**Key words**

Fibrinolytic therapy – Tissue-type plasminogen activator – Acute myocardial infarction – Percutaneous transluminal coronary angioplasty – Fibrin metabolism

**Summary**

In order to define some of the determinants of successful thrombolysis and reocclusion during fibrinolytic therapy for acute myocardial infarction (AMI), specific molecular markers of fibrin metabolism were serially measured in 15 patients with AMI treated with tissue-type plasminogen activator (t-PA). Fibrin formation was assessed by measurement of fibrinopeptide A (FpA) and fibrinolysis by assay of B-β peptides 1–42 and 15–42 and crosslinked fibrin degradation products (XDP). At baseline, FpA levels were high while markers of fibrinolysis were near normal. Following a 90-minute infusion of t-PA (0.5–1.1 mg $kg^{-1}$ $hr^{-1}$), all markers of fibrinolysis increased. Levels of FpA remained elevated despite heparin at the initiation of cardiac catheterization. None of these markers discriminated between patients with successful reperfusion from those without. At 4 hours, B-β 15–42 peptide and XDP levels remained elevated suggesting persistence of fibrinolysis beyond the short circulatory half-life of t-PA. FpA levels at 4 hours were lower in patients who underwent acute coronary angioplasty compared to those who received additional low dose t-PA (12.3 ± 4.5 vs. 30.4 ± 5.5 ng/ml, $p < 0.05$). By 48 hours, markers of fibrinolysis had returned toward normal except in 2 patients with persistently elevated B-β 15–42 peptide levels who suffered reocclusion on days 5 and 6 (75 and 44 vs. 29 ± 3 nM, $p < 0.005$). In conclusion, molecular markers of fibrin metabolism during fibrinolytic therapy may provide clinically relevant data.

## Introduction

Although fibrinolytic therapy administered early during the course of acute myocardial infarction appears to be beneficial, a significant number of patients are not successfully reperfused or suffer early reocclusion (1–4). The factors influencing the success of fibrinolytic therapy are not well understood but probably ultimately depend on the balance between ongoing thrombosis and fibrinolysis. These processes predominantly reflect the balance between thrombin promotion of clot formation and plasmin-mediated fibrinolysis. The initial step in the conversion of fibrinogen to crosslinked fibrin polymer involves the cleavage of fibrinopeptide A (FpA), by thrombin from the A-α chain of fibrinogen, to yield fibrin I (5). If plasmin activity is intense, degradation of fibrin I occurs, yielding a number of peptide fragments including B-β 1–42 peptide (derived from the B-β chain of fibrin/fibrinogen) which is specific for degradation of either fibrin I or fibrinogen (Fig. 1). If thrombin activity dominates, fibrin II is generated by the removal of fibrinopeptide B, from the B-β chain. Plasmin mediated proteolysis of fibrin II yields B-β 15–42 peptide which is specific for fibrinolysis of fibrin II. In the absence of local plasmin activity, fibrin will polymerize and covalent bonds between γ and α chains will be catalyzed by thrombin activated factor XIIIa to form crosslinked fibrin polymer (6).

The development of a radioimmunoassay for FpA by Nossel and coworkers has allowed assessment of in vivo thrombin activity (7). Due to FpA's half-life of 3–5 minutes (8), levels of plasma FpA are indicative of ongoing thrombin activity (8–10). Until recently, assessment of fibrin degradation products has been compromised by assays which did not discriminate between degradation products of fibrin and those of fibrinogen (11). This represents a particularly difficult problem for studying fibrin metabolism during fibrinolytic therapy since all currently available fibrinolytic agents are not completely fibrin specific and cause some systemic fibrinogenolysis as well (12). Recently, Kudryk and coworkers have developed specific monoclonal antibodies for both the B-β 1–42 and 15–42 peptides which have been incorporated into immunoassays (13, 14). Similarly, an immunoassay for crosslinked fibrin degradation products (XDP) has been recently developed utilizing a monoclonal antibody raised against the unique antigenic characteristics of the γ–γ crosslinks at the D-D region of fibrin (15, 16). Previous clinical studies with this assay have documented elevated XDP levels in disseminated intravascular coagulation, metastatic cancer and after streptokinase treatment of deep venous thrombosis and pulmonary embolism (17, 18).

The purpose of this study was to assess thrombin and plasmin activity using specific molecular markers of fibrin metabolism in patients with acute myocardial infarction treated with recombinant tissue-type plasminogen activator (t-PA) including a subgroup of patients undergoing acute percutaneous transluminal coronary angioplasty. On-going thrombin activity was assessed by measurement of FpA levels and fibrinolysis was studied by measurement of B-β 1–42 and 15–42 peptides and XDP.

## Patients, Materials and Methods

*Clinical Protocol*

We studied 15 patients with acute myocardial infarction within 6 hours of the onset of chest pain and with at least 0.2 mV ST segment elevation in 2 or more ECG leads. Patients were excluded for age greater than 75

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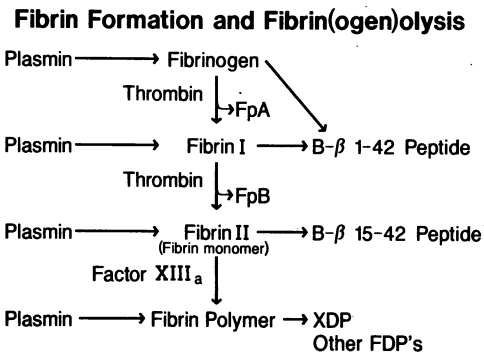


*Fig. 1* Schematic of fibrin(ogen) metabolism. FpA = fibrinopeptide A; FpB = fibrinopeptide B, XDP = crosslinked fibrin degradation products; FDPs = fibrin(ogen) degradation products

years, previous cardiac surgery, cardiogenic shock, or contraindications to thrombolytic therapy.

As part of an open label multicenter study protocol, patients received an intravenous infusion of recombinant double-chain t-PA (Burroughs Welcome Co., Research Triangle Park, NC) at a dose of 0.5–1.1 mg $kg^{-1}$ $hr^{-1}$ for 90 minutes. All patients underwent urgent cardiac catheterization shortly after initiation of the t-PA infusion. Heparin 5,000 U was administered after insertion of the vascular sheaths. Flow in the infarct related vessel at the end of the 90 minutes was graded according to the TIMI criteria (0 = no flow, I = penetration of contrast dye without perfusion, II = partial perfusion, III = complete perfusion) (1). Patients with TIMI grade II or III flow of the infarct artery received an additional 90-minute infusion of t-PA at one-third the initial dose unless coronary angioplasty was performed at 90 minutes at the discretion of the attending cardiologist. Patients with TIMI grade 0 or I perfusion at 90 minutes underwent urgent attempted angioplasty of the infarct vessel. All patients received additional heparin for at least 72 hours to maintain their partial thromboplastin time at approximately twice control. In order to evaluate the effects of cardiac catheterization and angioplasty on indices of fibrin formation and degradation, pre and post procedure samples were also obtained in patients with unstable angina and post-infarction angina who had not been treated with continuous heparin or fibrinolytic agents.

*Sample Collection and Processing*

Samples were obtained immediately prior to initiation of t-PA (baseline), at 90 minutes, 4, 24 (range 18–27 hours), and 48 hours (range 44–49 hours) and at 7–10 days. Blood was collected by careful venipuncture or through a femoral venous sheath (after discarding the first 4–5 ml of blood) and immediately placed into a prechilled tube containing an anticoagulant solution of heparin 1,000 U/ml, aprotonin 900 KIU/ml and ethylenediaminetetraacetic acid 10 mM at a ratio of 9 volumes blood to 1 volume anticoagulant. The tubes were maintained in an ice bath for 15 minutes followed by centrifugation at 1,500 g for 15 minutes and the plasma was immediately frozen at −40° C until assay.

In order to assess the degree of in vitro artifactual elevation of the fibrin markers generated by the presence of t-PA during sample processing and storage, we added t-PA (provided by Burroughs Wellcome Co.) at a final concentration of 2.5 μg/ml into blood from normal subjects and processed them as described above. This dose of t-PA was based on approximate peak plasma values expected for patients treated with t-PA (19).

*Assay Methods*

Plasma for FpA determination was treated with bentonite to remove fibrinogen. FpA was measured by enzyme linked immunosorbent assay (ELISA) using a commercially available kit (Diagnostica Stago, Asnières-Sur-Seine, France). Standards and bentonite treated samples were incubated overnight with anti-FpA rabbit antibody and added for 1 hour to a micro-titer plate (Costar 3690, Van Nuys, CA) coated with synthetic FpA. After washing, anti-IgG antibody horseradish peroxidase conjugate was added followed by orthophenylenediamine peroxide color substrate and the reaction was terminated with sulphuric acid. Absorbance at 492 nm read with a Titertek plate reader (Flow Laboratories, Rockville, MD). Normal values for FpA in our laboratory are 2.4 ± 0.3 ng/ml (mean ± SEM).

Plasma for B-β 1–42 peptide was centrifuged in a Centricon-30 microconcentrator (Amicon, Danver, MA) at 1,500 g for 60 minutes at 4° C. The resultant ultrafiltrate was used for B-β 1–42 peptide determination. Plasma samples for B-β 15–42 peptide were heated at 56° C for 30 minutes to denature and precipitate fibrinogen which would otherwise interfere with the assay. Both peptides were measured with commercial ELISA kits (New York Blood Center, New York, NY). For the B-β 1–42 peptide assay, a Falcon Probind 3915 microtiter plate (Becton, Dickinson & Co, Oxnard, CA) was coated with fibrinogen while the B-β 15–42 peptide assay utilized a Costar 2596 microtiter plate which was coated with fibrin II monomer. Prepared samples and standards were mixed with a specific monoclonal antibody. In the B-β 1–42 peptide assay, the antibody reacts with fibrinogen as well as B-β 1–42 peptide. The B-β 15–42 peptide assay employs an antibody which recognizes fibrin II in addition to B-β 15–42 peptide. After 30 minutes incubation, the plates were washed and anti-IgG-horseradish peroxidase conjugate was added for 45 minutes. ABTS peroxide color reagent was added and the reaction was terminated at 20 minutes with 5% sodium dodecyl sulfate. Absorbance at 404 nm (reference wavelength 450 nm) was determined in the plate reader. Normal values in our laboratory for B-β 1–42 and 15–42 peptides are 4.5 ± 0.7 nM and 20.8 ± 1.7 nM respectively.

Determination of XDP plasma levels were performed using another commercially available ELISA kit (American Diagnostica, Greenwich, CT). A Falcon Probind 3915 microtiter plate was coated with a monoclonal antibody (DD-3B6) to the γ–γ crosslinks at the D-D region of fibrin. Samples and standards were added to the plate for 60 minutes and washed. A second antibody (DD-4D2) conjugated to horseradish peroxidase was added for 60 minutes and washed. ABTS color reagent was added for 20 minutes and the reaction terminated by sodium fluoride. Absorbance at 415 nm was determined. Normal plasma XDP values are 60 ± 6 ng/ml.

*Statistics*

The results are expressed as mean ± standard error of the mean. In comparing fibrin markers between groups, data was analyzed by the paired and unpaired Student's t-test as appropriate. The relationship between t-PA dose and levels of each marker were examined using linear regression techniques. A p-value $<0.05$ was considered significant.

## Results

*Clinical Observations*

The clinical characteristics of the study group are summarized in Table 1. All patients demonstrated significant elevation of serum creatinine kinase MB isoenzyme levels consistent with the diagnosis of acute myocardial infarction. Reperfusion at 90 minutes (TIMI grade II or III) occurred in 12 of 15 (80%) of patients. Angioplasty was attempted in 7 patients at the end of the initial 90-minute t-PA infusion. No additional heparin was given prior to angioplasty. Three of these patients had totally occluded infarct vessels at 90 minutes while 4 patients had TIMI grade II or III flow. Angioplasty was successful in 6 of these 7 patients, however, one (with an inferior myocardial infarction) developed cardiac tamponade from perforation of the right ventricle by a pacing catheter and underwent surgery emergently. Another patient who had TIMI grade III flow at 90 minutes and received the additional 90-minute lower dose t-PA infusion developed reocclusion of his infarct related artery 15 minutes after termination of the second infusion also underwent urgent successful coronary angioplasty. This patient also received heparin 4,000 U and streptokinase 50,000 U intracoronary prior to angioplasty. An additional 2 patients (including a patient with initially successful angioplasty) developed chest pain, ECG changes and angiographically documented reocclusion on days 5 and 6. Two

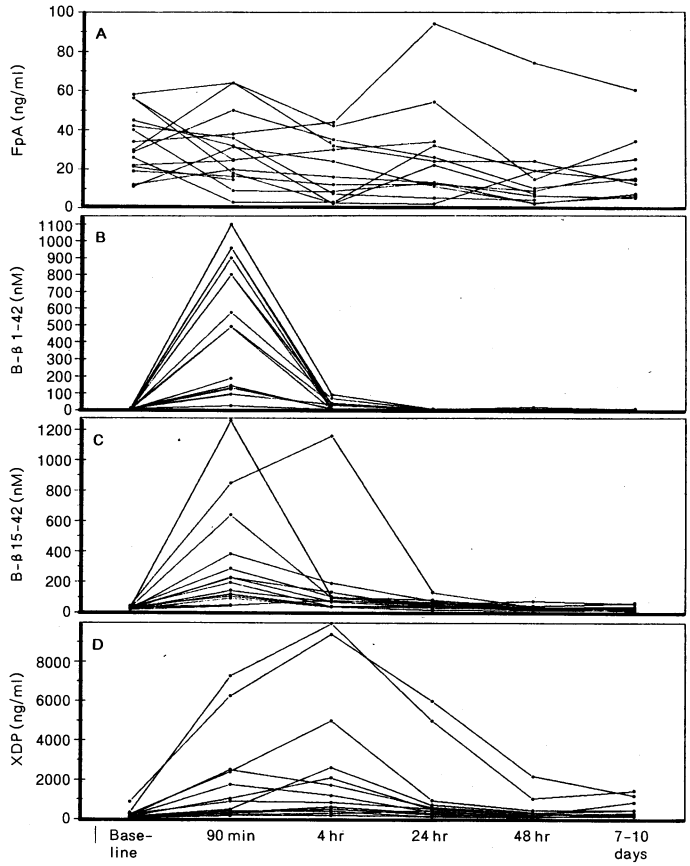


*Fig. 2* Levels of fibrinopeptide A (FpA), B-β 1–42 peptide, B-β 15–42 peptide, and crosslinked fibrin degradation products (XDP) measured in each patient at baseline, 90 minutes, 4, 24, and 48 hours and 7–10 days

patients underwent coronary artery bypass surgery at 2 and 36 hours respectively. Results obtained after surgery were not included in the data analysis. The 7–10-day sample in another patient was not collected.

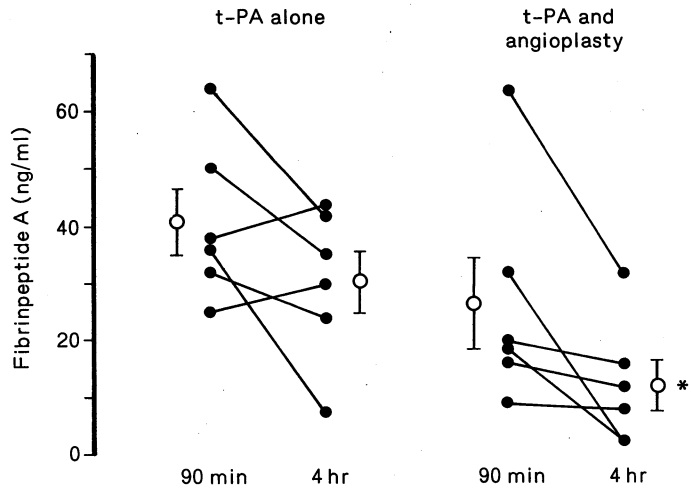


*Fig. 3* Fibrinopeptide A levels at 90 minutes and 4 hours in patients treated with an additional low dose infusion of t-PA (shown on left) versus patients who underwent acute successful coronary angioplasty (shown on right). * = $p < 0.05$ vs. 90-minute preangioplasty levels and 4-hour low dose t-PA levels

*Fibrinopeptide A Levels*

The results obtained for FpA levels are shown in Fig. 2A. Baseline levels of FpA, prior to initiation of t-PA therapy, were markedly elevated at 33.5 ± 3.9 ng/ml. After the completion of the initial 90-minute t-PA infusion, FpA levels remained elevated in general (29.8 ± 4.6 ng/ml) and decreased to below 10 ng/ml in only 2 patients despite the administration of 5,000 U heparin 45 ± 10 min previously. In the 3 patients who did not reperfuse (TIMI grade 0) at the end of the 90-minute t-PA infusion, FpA values at 90 minutes were 3.2, 18.4, and 64.0 ng/ml. At 4 hours, FpA levels dropped slightly to 19.9 ± 4.1 ng/ml, but remained appreciably above normal at 24 and 48 hours (26.3 ± 6.6 and 15.9 ± 5.4 ng/ml respectively) despite a continuous heparin infusion. FpA levels remained high in most patients during convalescence at 7–10 days (19.8 ± 5.0 ng/ml).

The effect of coronary angioplasty on FpA levels at 4 hours was significant (Fig. 3). In the 6 patients who underwent successful angioplasty prior to this sampling point, FpA levels were significantly lower (12.3 ± 4.5 ng/ml) than in the 6 patients who had TIMI grade III flow at 90 minutes and received an additional 90-minute t-PA infusion (30.4 ± 5.5 ng/ml, $p < 0.05$). Interestingly, the patient with the highest FpA value (32.0 ng/ml) at 4 hours experienced reocclusion of the infarct artery on day 5 after having had a successful acute angioplasty.

*B-β 1–42 and 15–42 Peptide Levels*

The results for these fibrinopeptides are shown in Figs. 2B and 2C. The baseline values for B-β peptide 1–42 (5.5 ± 0.6 nM) and for B-β 15–42 peptide (26.1 ± 1.9 nM) were only minimally elevated over normal. However, at the end of the initial 90-minute t-PA infusion, marked elevations of both peptides were detected. B-β 1–42 peptide concentration increased almost 100-fold to 519.0 ± 94.6 nM, while the increase in B-β 15–42 peptide was approximately 10-fold to 315.5 ± 86.6 nm. There was no apparent relationship of the t-PA dose administered to the observed levels of either of these peptides. At 4 hours, B-β 1–42 peptide concentration declined to 26.0 ± 7.6 nM, was normal by 24 hours (4.7 ± 0.4 nM) and remained unchanged at 48 hours (5.9 ± 1.0 nM) and at 7–10 days (5.1 ± 0.5 nM). In contrast, B-β 15–42 peptide concentration at 4 hours declined by only half to 166.8 ± 80.4 nM and at 24 hours was still over twice baseline at 58.4 ± 7.9 nM. By 48 hours, the B-β 15–42 peptide concentration had returned to near baseline exept for 2 patients who developed reocclusion of their infarct artery at days 5 and 6 (Fig. 4). The 48-hour B-β 15–42 peptide concentrations in these 2 patients were significantly elevated (75 and 44 nM) compared to the patients without subsequent reocclusion (29 ± 3 nM, $p < 0.005$). At 7–10 days, the B-β 15–42 peptide concentration (32.9 ± 4.7 nM) remained approximately 50% higher than controls.

*Crosslinked Fibrin Degradation Product Levels*

Levels of XDP at baseline were only minimally increased at 194.0 ± 51.3 ng/ml compared to normal control ($p < 0.01$). Fig. 2D shows the time course of XDP values after t-PA therapy. Following the initial 90-minute t-PA infusion, XDP levels rose to 1,631.5 ± 556.0 ng/ml. XDP levels did not predict successful response to therapy at 90 minutes as nonresponders had XDP values ranging from 416 to 7,262 ng/ml. As was the case with the B-β peptides, there was no observed relationship between the t-PA dose and XDP levels. At 4 hours, XDP levels continued to rise to 2,694.2 ± 901.4 ng/ml, significantly higher ($p < 0.05$) than values at 90 minutes even though additional t-PA had not been administered for 60–150 minutes previously. There was no

difference in XDP values at 4 hours between patients who received the additonal 90-minute low dose t-PA infusion and those who did not (2,762.2 ± 1,251.5 vs. 2,365 ± 1,328.1 ng/ml respectively, p NS). By 24 hours, XDP values had declined to 1,233.4 ± 511.8 ng/ml and to 456.5 ± 166.2 ng/ml by 48 hours. An apparent increase in XDP levels at 7–10 days (567.4 ± 134.4 ng/ml) was not related to left ventricular thrombus as assessed by two-dimensional echocardiography.

*Effects of Catheterization on Fibrin Metabolism*

In order to assess the effects of catheterization on fibrin metabolism, we collected pre and post procedure samples from patients with unstable angina and post-infarction angina undergoing cardiac catheterization. None of these patients were on a heparin maintenance drip but did receive 3,000–5,000 U heparin at the beginning of the procedure. FpA levels (n = 8) were initially elevated at 41.1 ± 11.8 ng/ml in this group but declined to 7.6 ± 3.0 ng/ml ($p < 0.01$) at the end of the procedure consistent with the known effects of heparin on FpA formation (18). Fibrinolysis as assessed by XDP levels (n = 11) were essentially unchanged pre and post procedure (138 ± 25 vs. 128 ± 28 ng/ml respectively, p NS).

*Assessment of Possible In Vitro Artifact*

In order to assess the possibility of in vitro t-PA mediated proteolysis, we treated blood from normal subjects (n = 5) with t-PA as described in the Methods section and measured B-β 1–42 peptide levels. The addition of 2.5 μg/ml of t-PA caused a significant ($p < 0.001$) elevation of B-β 1–42 peptide levels to 106 ± 27 nM (normal <6.7 nM). Since the values for B-β 1–42 peptide levels to 106 ± 27 nM (normal <6.7 nM). Since the values for B-β 1–42 peptide from patient samples at 90 minutes were typically in excess of 490 nM, it seems likely that most of this measured peptide represents in vivo generation, however this cannot be conclusively derived from the available data. FpA, B-β 15–42 peptide and XDP levels were not appreciably affected by addition of t-PA.

*Table 1* Clinical characteristics

| | |
|---|---|
| No. of patients | 15 |
| Age (years) | 58.9 ± 2.6 |
| Sex (male/female) | 11/4 |
| Time from onset chest pain to initiation of t-PA (minutes) | 191 ± 18 |
| Infarct artery (No.) | |
| left anterior descending | 5 |
| right | 10 |
| Dose t-PA (No.) | |
| 0.5 mg $kg^{-1}$ $hr^{-1}$ | 4 |
| 0.6 mg $kg^{-1}$ $hr^{-1}$ | 3 |
| 0.7 mg $kg^{-1}$ $hr^{-1}$ | 3 |
| 0.9 mg $kg^{-1}$ $hr^{-1}$ | 3 |
| 1.1 mg $kg^{-1}$ $hr^{-1}$ | 2 |
| Perfusion status of infarct artery at 90 minutes | |
| TIMI grade 0 | 3 |
| TIMI grade I | 0 |
| TIMI grade II | 2 |
| TIMI grade III | 10 |
| Acute angioplasty (successful) | 8(7) |
| Acute cardiac surgery | 2* |

* Includes one patient who also underwent acute angioplasty.

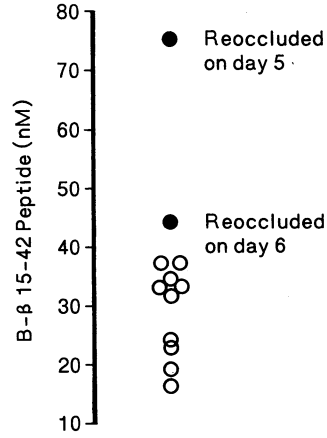


*Fig. 4* B-β 15–42 peptide levels at 48 hours. ● = patients who experienced reocclusion, ○ = patients without reocclusion

## Discussion

*Fibrin Metabolism at Baseline During Acute Myocardial Infarction*

During the first few hours of myocardial infarction, thrombin activity was increased as reflected by markedly elevated FpA levels as previously reported (20–24). We also confirmed previous studies that XDP and B-β 1–42 and 15–42 peptide levels are only minimally elevated over normal in most patients with acute myocardial infarction (24–26). This suggests that during the early stages of myocardial infarction, thrombin activity predominates and that plasmin-induced fibrinolysis is inadequate.

*Fibrin Metabolism During and Early After Treatment with Tissue Plasminogen Activator Therapy*

At the conclusion of the initial 90-minute t-PA infusion, all markers of fibrinolysis rose markedly, consistent with activation of plasminogen to plasmin by t-PA. The predominant fibrin degradation marker measured at this time period was B-β 1–42 peptide. This peptide may be generated from proteolysis of fibrin I or circulating fibrinogen to yield fragment X, which when acted on by thrombin forms a weaker clot than those formed from fibrinogen (27, 28). This peptide may be formed in vivo or as a post sampling artifact. Since the concentration of B-β 1–42 peptide measured in clinical samples were over 5-fold greater than in the in vitro t-PA incubated samples, we believe that the majority of measured B-β 1–42 peptide in the study samples was generated in vivo. Similar results with B-β 1–42 peptide measured in patients who received either t-PA or streptokinase have also recently been reported (29). In addition to the marked increase in B-β 1–42 peptide levels at 90 minutes, large increases in B-β peptide and XDP were noted indicating active fibrinolysis of fibrin II and crosslinked fibrin. There was no correlation in our study between the dose of t-PA and the degree of fibrinolysis as assessed by these markers, although a much larger number of patients may be necessary to demonstrate a relationship.

Previous studies with these markers during acute myocardial infarction treated with streptokinase or acetylated plasminogen: streptokinase activator complex have also demonstrated generation of large quantities of fibrin degradation products (24, 30). As in our study, XDP levels did not discriminate responders from nonresponders. Seifried and colleagues reported an increase in fibrin degradation products in healthy volunteers following administration of t-PA (31). This finding suggests that XDP may originate from lysis of systemic fibrin and is not specific for intracoronary fibrinolysis.

Eisenberg and coworkers reported increasing XDP levels in patients with acute myocardial infarction for several hours after discontinuation of t-PA therapy (32). This is in keeping with our findings and suggests that fibrinolysis persists beyond the short circulatory half-life of t-PA (approximately 5 minutes), although elevated XDP values at 4 hours are at least partially due to its prolonged half-life (33). Persistence of fibrinolysis is also suggested by the elevated B-β 15–42 peptide levels at 4 hours. Since B-β 15–42 peptide has a half-life of only 10–20 minutes, elevation of this peptide reflects ongoing fibrinolysis (33). Whether this reflects a prolonged activity phase of t-PA or stimulation of endogenous fibrinolytic mechanisms is not clear from this data.

In addition to the increase in markers of fibrin(ogen)olysis following administration of t-PA, thrombin activity, reflected by FpA formation, remained high in most patients despite treatment with heparin. This finding is in contrast to the consistent decrease in FpA levels in patients with acute myocardial infarction following heparin treatment (20). The reason for continued FpA formation in t-PA treated patients despite heparin is unclear but may be partially due to a particularly intense thrombogenic nidus at the site of the coronary plaque rupture. Fibrinolytic therapy removes recently formed thrombus but in doing so may leave the plaque rupture site continuously exposed so that it serves as a nidus for further thrombus formation. While increased FpA levels following administration of streptokinase in patients who did not reperfuse and decreased levels in those successfully recanalized has been reported (34), we did not find any differences in FpA levels between responders and nonresponders in our t-PA treated patients. FpA levels remained high at 4 hours in those patients who received additional low dose t-PA and heparin, but decreased in patients who had succesful angioplasty. Although the number of patients in this nonrandomized study who underwent coronary angioplasty is small and there was some overlap in FpA levels between those who underwent angioplasty and those who did not, it is somewhat unexpected that mechanical intervention within the coronary artery would actually decrease ongoing thrombosis. This suggests that vessel remodeling and/or improved flow through the infarct-related artery by angioplasty may make the lesion less thrombogenic. Interestingly, the one patient with a persistently elevated FpA level after angioplasty suffered late reocclusion.

### *Fibrin Metabolism Late (24 Hours and Later) Following t-PA Therapy*

At 24 hours and beyond, B-β 1–42 peptide levels had returned to and remained at baseline. However, B-β 15–42 peptide and XDP levels, although decreased from 4 hours, remained elevated over baseline indicating persistent fibrinolytic activity. Concurrently, new fibrin formation as evidenced by elevated FpA values was occurring despite therapeutic heparin infusions. By 48 hours, all markers had returned or were returning towards the normal range except in 2 patients with elevated B-β 15–42 peptide levels who experienced reocclusion on days 5 and 6. It should be emphasized that only 2 patients suffered late reocclusion and that further studies will be necessary to confirm and expand on this finding. For unknown reasons, there was a relatively small increase in FpA and XDP levels at 7–10 days. Possible etiologies for this increase include remodeling of the intracoronary thrombus site, clinically occult deep venous thrombosis and/or ventricular thrombus.

### *Study Limitations*

A major potential problem in studying these markers is the possibility of in vitro activation of the coagulation system, especially in the presence of fibrinolytic agents. The addition of 2.5 μg/ml of t-PA to normal blood resulted in significant artifactual generation of B-β 1–42 peptide which is consistent with previous in vitro studies (29, 34). Since there was no appreciable concurrent elevation of FpA indicating fibrin I generation, the formation of B-β 1–42 peptides in the t-PA incubated blood specimens presumably reflects extensive t-PA mediated fibrinogenolysis. Recent studies have shown that the addition of D-phe-pro-arg-chloromethylketone (PPACK) (34) or antibody to t-PA (35) in the anticoagulant solution inhibits in vitro artifactual fibrinogenolysis. Even though it appears from this and previous studies (29) that most of the marked elevation of B-β 1–42 peptide at 90 minutes represents in vivo generation of this peptide, additional studies with an anticoagulant containing t-PA inhibitor (PPACK or t-PA antibody) are necessary for confirmation.

Another possible source of artifact is the introduction of catheters into the vasculature which may induce fibrin formation as reflected by elevation of FpA (36). We found that in a population of patients undergoing catheterization or angioplasty with high baseline FpA levels, FpA levels decreased substantially at the end of the procedure, presumably due to heparin pretreatment. Thus, it is unlikely that FpA formation in t-PA treated patients was due to catheter related fibrin formation.

Finally, while the number of patients in this study is small, it is clear from our data that although all patients with acute myocardial infarction treated with t-PA exhibit evidence of increased fibrin metabolism (both formation and degradation), there is a fairly wide variation in the magnitude of this rise. Nonetheless, we believe that these preliminary data provide new insight into the problems of failed fibrinolysis and reocclusion, and warrant further clinical studies with these molecular markers.

### *Clinical Implications*

The use of fibrinolytic therapy during the early stages of acute myocardial infarction results in marked, but variable, effects on fibrin metabolism regardless of whether or not reperfusion of the infarct artery occurs. Even after successful thrombolysis, indices of both fibrin formation and degradation remain elevated in most patients despite heparin. In patients who also underwent acute coronary angioplasty, ongoing fibrin formation, as reflected by FpA levels, was significantly reduced when compared to those patients with patent arteries who did not receive angioplasty. This suggests that vessel remodeling achieved by angioplasty may stabilize the intraluminal lesion and/or improves flow, making it less likely to act as a nidus for new fibrin formation. The clinical significance of this finding awaits further studies. Another observation was that the two patients who experienced late reocclusion had the highest concentrations of B-β 15–42 peptide at 48 hours. If this finding can be confirmed in larger clinical trials, B-β 15–42 peptide may be a useful marker to identify patients at increased risk for reocclusion following fibrinolytic therapy. In conclusion, we believe that specific molecular markers of fibrin formation and degradation are useful in assessing fibrin metabolism during fibrinolytic therapy and may provide a noninvasive means to guide the adequacy of therapy for these patients.

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