Thrombolytic Therapy With Tissue Plasminogen Activator or Streptokinase Induces Transient Thrombin Activity

By John Owen, Kenneth D. Friedman, Betty A. Grossman, Carol Wilkins, Andrew D. Berke, and Eric R. Powers

We have determined the plasma level of fibrinopeptide A as a specific index of thrombin activity during the infusion of a thrombolytic agent in patients with acute myocardial infarction. Peripheral venous plasma levels of fibrinopeptide A increased following the initiation of thrombolytic therapy from 2.7 nmol/L to a peak of 13.0 nmol/L at 30 minutes with streptokinase and from 1.1 nmol/L to a peak of 10.7 nmol/L at 90 minutes with tissue plasminogen activator. The amount of fibrinogen converted to fibrin I was determined by integration of the plasma level of fibrinopeptide A over time. The amount of fibrin I formed over the five-hour period from the start of drug infusion was approximately 10 mg/dl in response to either streptokinase or recombinant tissue plasminogen activator. We conclude that activation of coagulation occurs in response to thrombolytic therapy despite heparin administration. This thrombin action, though transient, would be sufficient to cause rethrombosis if localized and incompletely opposed by fibrinolytic activity.

A CUTE MYOCARDIAL INFARCTION is a major cause of morbidity and mortality in Western societies. Data presented during the past few years have strongly supported the concept that coronary artery thrombosis is a major cause of acute myocardial infarction. One of the problems in studying this disorder is the difficulty of observing the actual thrombotic event. Studies in this institution of patients at increased risk for myocardial infarction have generally yielded negative results. In a series of studies of patients with coronary artery disease we have attempted to demonstrate intracoronary coagulation activity. We did not observe such activity either at rest, in response to exercise, or in response to pacing-induced myocardial ischemia. It appears therefore that angina induced by increased myocardial oxygen demand is not a good model of the thrombotic events leading to acute myocardial infarction.

Studies in animals and humans have shown that the myocardium can survive a period of ischemia but that the damage is proportional to the duration of ischemia. Such observations led to the use of thrombolytic agents as a means of restoring blood flow in patients with acute myocardial infarction. Thrombolytic therapy has proved to be successful in restoring blood flow to the myocardium. Recently a large multicenter trial of thrombolytic therapy with streptokinase (SK) was carried out in Italy. The study showed that thrombolytic therapy in the acute setting significantly reduced the mortality from acute myocardial infarction.

A major problem in the use of thrombolytic agents has been the occurrence of thrombotic reocclusion of the coronary artery. Heparin administration has been used to minimize this problem; however, a significant proportion of patients have been noted to suffer rethrombosis of the affected coronary artery despite apparently adequate blood levels of heparin. Data from studies of the duration of infusion suggest that there is a high incidence of rethrombosis following one hour of tissue-type plasminogen activator (t-PA) infusion but that the incidence markedly declines when the duration of infusion is increased. These observations suggest that, in response to the infusion of a thrombolytic agent, there is activation of the coagulation system. This coagulant activity is not fully inhibited by heparin administration, but the activity is in some way self-limited. One postulate is that thrombogenic material is exposed by the dissolution of the thrombus.

Thrombin action on fibrinogen is essential for thrombus formation. In the initial reaction thrombin cleaves two moles of fibrinopeptide A (FPA) from each fibrinogen to form 1 mol of fibrin I. The plasma level of FPA thus specifically and quantitatively reflects in vivo thrombin action and fibrin I formation. The plasma level of FPA can be measured by radioimmunoassay, providing a highly sensitive measure of in vivo thrombin action. The short half-life of FPA in the circulation, about two minutes, makes this peptide ideal for investigating transient activation of coagulation and precisely determining the time of initiation of coagulation activity. We have used FPA to study thrombin activation in patients with acute myocardial infarction undergoing thrombolytic therapy with either SK or with t-PA.

MATERIALS AND METHODS

Subjects for this study were solicited from patients enrolled from this institution in the thrombolysis in myocardial infarction (TIMI) trial, which was carried out under the auspices of the National Heart, Lung, and Blood Institute. All patients eligible for the TIMI trial were considered eligible for the adjunct study of thrombin activity. Patients were admitted to the TIMI trial within seven hours of the onset of chest pain. Drug infusion was started within nine hours of the onset of chest pain. All subjects had detailed coronary angiographic studies after being anticoagulated with an intravenous (IV) bolus dose of 5,000 units of heparin. Once diagnostic images had been obtained, infusion of the drug was begun. The drug was administered through two intravenous (IV) lines: in one line was either recombinant t-PA (rt-PA) or placebo; in the other was SK or placebo. Each patient received one active drug and one placebo.

SK was given in a dose of 1,500,000 units as a constant infusion over 60 minutes. A total dose of 80 mg of t-PA was given over 3 hours at two different infusion rates: 40 mg/h for the first hour, then 20
mg/h for two hours. A continuous IV infusion of 1,000 U/h of heparin was begun one hour after the beginning of thrombolytic therapy. All catheters were regularly flushed with saline containing 1 U/mL heparin.

A heparin-bonded catheter lying in either the pulmonary artery or the right atrium was used to draw blood. After angiography, samples were collected immediately before starting the thrombolytic drug infusion, at intervals during the three-hour infusion, and then two hours after infusion. At each time point 9 mL of blood was withdrawn and added to 1.0 mL of anticoagulant containing heparin (1,400 U/mL), trasylov (1,000 U/mL), adenosine (10 mmol/L) and theophylline (20 mmol/L). Samples were placed on melting ice immediately after collection and held for not more than 60 minutes before further processing. Each sample was centrifuged at 3,000 g for 15 minutes and the plasma was transferred to another tube and centrifuged at 40,000 g for 20 minutes to remove platelets. In preparation for assay of FPA, fibrinogen and high-molecular weight (mol wt) degradation products were removed by ethanol precipitation. Three volumes of ethanol were added to 1 vol of plasma with constant mixing. After incubation at room temperature for 30 minutes the precipitate was deposited by centrifugation at 4,000 g for ten minutes. A measured aliquot of the supernate was transferred to a glass tube and taken to dryness in a Savant (Farmingdale, NY) Speed Vac. The residue was redissolved in 1/5 vol of distilled water, i.e. the original plasma volume.

Each blood sample was assayed for the plasma concentration of FPA by a double-antibody variation of our published radioimmunoassay procedure. For each patient, the concentration of rt-PA was determined in the 60-minute blood sample by using the enzyme-linked immunosorbent assay kit marketed by American Diagnostica (Greenwich, CT).

From the measurements of FPA were withheld from the primarily TIMI physicians, and the identity of the active agent employed was not revealed. The assays were performed without knowledge of either the clinical result of thrombolytic therapy or the identity of the active agent.

Cumulative fibrin I formation during the five hours following the initiation of thrombolytic drug administration was determined by piecisewise integration of the plasma FPA-v-time curve and by using a half-disappearance time of two minutes. Estimates of the reliability of these calculations were made by Monte Carlo simulation. The observed mean FPA concentrations were replaced by values randomly drawn from populations defined by the observed mean and SEM at each time point. The area under the curve was estimated by piecisewise integration and a value calculated for the amount of fibrin I formed. This random replacement procedure was carried out 10,000 times and the SD of the results used as an estimate of the variability of the calculated cumulative fibrin I formation.

The relationship between added thrombin and total amount of FPA released from fibrinogen in plasma was determined by adding human α-thrombin to citrated normal plasma. To a 1.0 mL aliquot of normal plasma was added 10 µL of a solution of human thrombin. The concentration of the thrombin solution was chosen to give final concentrations of 0.01 to 2.0 U/mL.

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RESULTS

Twenty-one patients were studied. All patients completed the investigative protocol of the primary TIMI trial. Eleven patients received rt-PA, and ten received SK. The mean plasma concentration of t-PA, 60 minutes after starting the infusion, was 996 ng/mL (range, 719 to 1,329) in patients receiving rt-PA and 7 ng/mL (range, 5 to 9) in patients who received SK. Assuming a volume of distribution of 2,500 mL, the calculated plasma half-disappearance times for rt-PA is approximately three minutes. This value implies that the steady state had been reached during rt-PA infusion.

The patients were grouped according to active drug received, and within groups the data were pooled according to time from start of the infusion. Plasma levels of FPA are shown in Fig. 1. The levels are shown as geometric means ± SEM. The closed symbols and solid line represent patients who received rt-PA, and the open symbols and broken line represent the patients who received SK. The first levels shown (1.1 nmol/L for rt-PA and 2.7 nmol/L for SK) are from samples collected after completion of angiography, immediately before administration of thrombolytic agent. The mean pretreatment level of all patients studied was 1.4 nmol/L, this level is within our normal range (0.2 to 1.6 nmol/L) despite the prior placement of multiple IV lines and
Teflon sheaths. In response to SK administration there is a rapid increase in FPA concentration to 13.0 nmol/L at 30 minutes; thereafter the level decreased. In response to rt-PA there was also a transient increase in the plasma level of FPA. In this case the increase was slightly delayed and reached a maximum of 12.0 nmol/L at 45 minutes. Analysis of variance of the FPA levels was carried out after logarithmic transformation of the data. There are highly significant differences from random variation: $P$ values less than $10^{-4}$ were calculated for the time course ($F = 37.25_{(9,135)}$), for the effect of the drugs ($F = 41.37_{(1,135)}$), and also for the difference in time course for the two drugs (the interaction term, $F = 24.07_{(9,135)}$).

A fractional clearance rate of 0.35/min, corresponding to a half-disappearance time of 2 minutes, was used to calculate the amount of fibrin I formation by integration of the FPA concentrations over time. These data are shown in Fig 2. The integral was determined by piecewise summation of the area under the FPA concentration–time curves separately for SK- and for t-PA–treated patients. The integrals were found to be 630 and 560 pmol/mL, respectively. Despite the highly significant differences in time course for the two drugs, the cumulated amount of fibrin I formed by thrombin action on fibrinogen was similar for the two agents. During the five hours from the start of the infusion, 10.7 ± 2.2 mg/dL of fibrinogen was converted to fibrin I in response to SK and 9.5 ± 1.3 mg/dL in response to rt-PA infusion.

The amount of plasma fibrinogen converted to fibrin I in vitro by exogenous thrombin acting in the fluid phase was determined from four independent experiments in the presence or in the absence of 1.0 U/mL heparin. These data are shown in Fig. 3. The two lines are essentially parallel; in the presence of 1.0 U/mL heparin some 30 times more thrombin is required to produce the same extent of FPA release. In the absence of heparin, 1 unit of thrombin releases approximately 9 μmol of FPA; in the presence of heparin, only 0.3 μmol of FPA is released by 1 unit of thrombin.

In the experiments examining the release of FPA by plasmin, we found that t-PA added to freshly prepared plasma was ineffective in releasing FPA, the rate of release being less than 5 pmol of FPA/μL plasma/μg t-PA. Sufficient plasmin was generated to cause the release of some 280 pmol of Bβ1-42/μL plasma/μg t-PA. In sharp contrast, when the same plasma was stored frozen before carrying out the experiment, there was the release of 180 pmol of FPA and 800 pmol of Bβ1-42/μL plasma/μg t-PA. In fresh plasma the initial rate of release of FPA in response to SK was 2.7 pmol of FPA/μL plasma/min/100 U SK. For each of the three doses studied (100, 200, and 400 U/μL) the total amount of FPA released reached a plateau at approximately 50 pmol/μL. These concentrations of SK were chosen on the basis of the plasma concentration expected for the rate of administration used in this study and a half-disappearance time from plasma of 85 minutes.

**DISCUSSION**

Our data demonstrate that there is transient elevation of the plasma FPA level in response to thrombolytic therapy (Fig 1), which we interpret as evidence of thrombin activity. From previous studies we would expect, in nonheparinized patients, that the placement of two peripheral IV lines and two Teflon vascular sheaths would lead to a circulating FPA concentration of greater than 10 nmol/L; thus the initial low level of FPA, <3.0 nmol/L, demonstrates that the heparin administered to our patients was active. In an unpublished study of 157 patients with acute myocardial infarction, we found a mean FPA level of 1.6 nmol/L, which supports our contention that acute myocardial infarction per se does not cause significant elevation of FPA levels. The close similarity in the observed curves from patients receiving SK and from
those receiving rt-PA suggests that the observation relates more to the class of therapy than to the individual agent. The peak FPA level occurs a little earlier for SK than for rt-PA and probably reflects the more rapid onset of action of SK compared with rt-PA.

The amount of FPA formed is a direct reflection of the amount of fibrin I formed. The amount of fibrinogen converted to fibrin I, over the five hours of this study, was approximately 10 mg/dL, or a total of 250 mg in an individual with a plasma volume of 2,500 mL. In vitro studies of the retraction of clots formed in normal platelet-rich plasma suggest that, without entrapment of RBCs, the volume of a clot formed from 250 mg of fibrinogen would be about 10 mL. This amount of fibrin clearly has the potential for producing a new thrombus; all that is required is localization and failure of the opposing lytic system. In these patients, we found that in response to SK and rt-PA plasmin action continued over the five hours of observation.

The amount of thrombin acting in the fluid phase needed to convert 10 mg/dL (0.3 nmol/mL) of fibrinogen into fibrin I in plasma can be estimated from the data shown in Fig 3. These in vitro data show that the release of 0.6 nmol/mL of FPA (1 mol of fibrinogen releases 2 mol of FPA) would require, in the presence of heparin, approximately 1.9 U/mL of thrombin. In the absence of heparin, 1.9 U/mL of thrombin could result in the cleavage of 30 times more FPA (equivalent to 300 mg fibrinogen/dL). The measured plasma fibrinogen concentration in these patients was 350 mg/dL, which suggests that the production of thrombin to a concentration of 1.9 U/mL would lead to almost complete conversion of fibrinogen to fibrin I in the absence of heparin. Eisenberg et al have measured FPA levels in a similar group of patients receiving SK without concomitant heparin anticoagulation. In their study they found that increasing levels of FPA during SK therapy correlated with the occurrence of rethrombosis. The marked elevations they observed, the highest being 2,403 ng/mL (1,566 nmol/L) support the hypothesis that large amounts of thrombin are released during thrombolytic therapy. However, the anticoagulant they used to collect the samples contained no heparin, so the possibility that there was significant ex vivo release of FPA cannot be ruled out. They also noted in their nonheparinized patients who were successfully reperfused that high pretreatment levels of FPA fell during thrombolytic therapy. This pattern was not observed in our patients, the majority of whom had successful reperfusion. In a subsequent paper Eisenberg et al reported finding a mean FPA concentration of 82.3 ng/mL (54 nmol/L) in similar patients before beginning thrombolytic therapy. This is in sharp contrast to our experience with 157 such patients who did not receive thrombolytic therapy and in whom we found a mean FPA concentration of 1.6 nmol/L.

The in vivo situation is surely more complex than that in vitro. An alternative explanation for the observed thrombin activity, despite heparin anticoagulation, is that in vivo the thrombin is insensitive to heparin-accelerated inhibition. The existence of such a pool of thrombin has been recognized to be present in both normal and pathological states. Administration of heparin to patients with thromboembolic disorders promptly normalizes the plasma FPA concentration but does not reduce the concentration to subnormal levels. In unpublished experiments we have confirmed the finding that heparin administered to normal volunteers does not significantly lower the plasma FPA level. We have observed, however, that patients receiving warfarin anticoagulation have subnormal levels of FPA, which supports the contention that the basal FPA concentration is the result of coagulation system activity.

Surface-bound thrombin could well account for this heparin resistance. It is plausible that the thrombus contributes to the elaboration of thrombin or that the vessel surface becomes exposed as a result of thrombolysis. These surfaces presumably had strong procoagulant properties only a short time earlier. Recent studies have shown that prothrombinase-catalyzed surface activation of prothrombin proceeds through the intermediate meizothrombin. This intermediate has some of the activity of thrombin, is not inhibited by antithrombin III/heparin cofactor, and retains the ability to bind to lipid surfaces. Clearly the influence of the surface on the activity of heparin would also serve to confound the calculations of the amount of thrombin needed to generate the observed FPA in vivo.

Another possibility is that the thrombus itself is the source of preformed active enzyme. Fibrin is known to bind and reversibly inactivate thrombin, and this thrombin can be liberated by plasmic digestion of the fibrin clot. The time course of the appearance of FPA suggests that lytic activity precedes thrombin action. However, the data do not allow us to choose directly between the alternative explanations for the observation that substantial thrombin activity was provoked by thrombolytic therapy in our patients.

We found that in fresh plasma in vitro there was virtually no release of FPA in response to plasminogen activation by t-PA. The amount of FPA released in vitro in response to SK was greater but would still account for less than 10% of the observed in vivo FPA release. These data suggest that in the fluid phase in vivo there would be little release of FPA in response to thrombolytic drug administration. In the identical experiments using t-PA and plasma that had been frozen, where FPA generation was found, the rate of cleavage of Bβ1-42 was noted to be markedly accelerated. This acceleration is consistent with the formation during freezing of a small amount of fibrin that in turn reversibly bound and inhibited the thrombin that caused its formation. This fibrin could then act as an accelerator of plasmin activation by rt-PA and also a source of thrombin to release FPA. While the possibility cannot be ruled out, the data obtained with fresh plasma suggest that plasmin-mediated FPA release in vivo is not likely to explain our observations. This interpretation is also consistent with the data of Takagi and Doolittle. They found, in a system containing only fibrinogen and plasmin, that the Ao16-17 bond was cleaved only as a late event in the complete digestion of fibrinogen.
lant forces that led to the coronary artery thrombosis responsible for myocardial infarction. These data, however, do not allow the explicit localization of the source of the FPA, and the precise mechanism for the transient nature of the thrombin action will require new data to fully elucidate. These results are compatible with the release of prothrombin from a thrombus undergoing dissolution or the elaboration of prothrombinase activity with subsequent inhibition. We believe that patients with acute myocardial infarction undergoing thrombolytic therapy may provide a model of coronary artery thrombogenesis, thus opening a window on the events leading up to coronary occlusion. This model may therefore be of great value in studies of the pathophysiology of acute myocardial infarction.

REFERENCES


Thrombolytic therapy with tissue plasminogen activator or streptokinase induces transient thrombin activity

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