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Activation of Prothrombin Accompanying Thrombolysis With Recombinant Tissue-Type Plasminogen Activator

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Increases in thrombin activity in patients given fibrinolytic agents for acute myocardial infarction have been shown to be important in limiting the ultimate success of coronary thrombolysis. The present study was designed to determine whether increases in thrombin activity reflect, in part, activation of prothrombin accompanying thrombolysis. Plasma concentrations of prothrombin fragment 1.2, a polypeptide released when prothrombin is activated by factor Xa, were measured in 22 patients with acute myocardial infarction before and after treatment with 100 mg of recombinant tissue-type plasminogen activator (rt-PA). Concentrations of prothrombin fragment 1.2 increased from 0.83 ± 1.1 nM (mean ± SD) before rt-PA infusion to 1.5 ± 1.5 nM 2 h after initiation of the infusion ($p < 0.05$). After a 5,000-U intravenous dose of heparin given at the end of the infusion of rt-PA, concentrations of prothrombin fragment 1.2 decreased from 1.8 ± 1.5 to 1.1 ± 0.9 nM ($n = 20$, $p < 0.05$), although values were still increased compared with concentrations before rt-PA.

These results indicate that thrombin activity increases in patients given rt-PA at least in part because of activation of the coagulation system leading to activation of prothrombin. Thus, inhibition of the reactions involving coagulant proteins that lead to activation of prothrombin may be of value as conjunctive treatment to potentiate the efficacy of pharmacologic thrombolysis.

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The role of persistent thrombin activity in inducing immediate or delayed thrombotic reocclusion in patients with myocardial infarction treated with fibrinolytic agents is now appreciated (1–5). However, mechanisms responsible for increases in thrombin activity accompanying thrombolysis have not been completely defined. We have shown that thrombin activity increases in vitro in response to activation of plasminogen with streptokinase or tissue-type plasminogen activator (rt-PA) as a consequence of plasmin-mediated activation of the coagulation system (6). In addition, thrombin bound to fibrin during formation of clots remains active and may be exposed in clots undergoing lysis, promoting continued fibrin formation, platelet activation and activation of the coagulation system (7,8). Lysis of clot may expose other procoagulant factors, such as tissue factor in the subendothelium, which can induce activation of the coagulation system. Although several pharmacologic strategies to inhibit coagulation during thrombolysis are possible, selection of optimal conjunctive anticoagulant regimens would be facilitated by knowledge of the mechanisms responsible for increased procoagulant activity during thrombolysis (9).

In patients given streptokinase or rt-PA without concurrent intravenous heparin, plasma concentrations of fibrinopeptide A, a marker of thrombin activity, increase markedly (4,10). Increases in the concentration of fibrinopeptide A are smaller when patients treated with fibrinolytic agents are given concurrent heparin, consistent with the inhibition of thrombin by heparin (11). Although increases in fibrinopeptide A reflect increased thrombin activity, the extent to which such increases are attributable to new elaboration of thrombin, as opposed to activity of preformed thrombin exposed during thrombolysis, cannot be determined by measurement of fibrinopeptide A. Recently, Gulba et al. (5) found that the concentration of thrombin-antithrombin III complexes increased in plasma of patients treated with rt-PA, consistent with an increase in the amount of thrombin available for inhibition by antithrombin III. However, the extent to which these results reflect activation of the coagulation system and consequent activation of prothrombin has not been defined. Because inhibition of earlier steps in the coagulation pathway that lead to activation of prothrombin may be more efficient than inhibition of thrombin in attenuating procoagulant activity, we sought to define the extent of prothrombin activation in patients treated with rt-PA for acute myocardial infarction by measuring changes in the plasma concentration of the prothrombin fragment 1.2,

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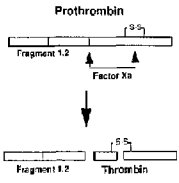

Figure 1. Schematic representation of the activation of prothrombin by factor Xa. Factor Xa cleaves the prothrombin molecule, releasing prothrombin fragment 1.2 and forming the active enzyme, thrombin, a two-chain molecule. Activity of factor Xa toward prothrombin is greatly increased when Xa is associated on phospholipid membranes with its cofactor, activated factor V (Va)(18).

a polypeptide that is released when thrombin is formed (12,13) (Fig. 1).

Methods

Study patients. Twenty-two patients with acute myocardial infarction who were admitted to the Cardiac Care Unit of Barnes Hospital at Washington University Medical Center and treated with rt-PA (Alteplase, Genentech) were studied. The decision to treat patients with rt-PA was based on clinical criteria, which generally included the presence of at least 0.1 mV of ST segment elevation in two anatomically contiguous leads on the admission electrocardiogram. Exclusion criteria were those accepted to be contraindications to the administration of fibrinolytic agents (14). Administration of rt-PA was initiated with a 6-mg bolus followed by an additional 54 mg over the 1st h; then 40 mg was given over 2 h as a 20-mg/h infusion. In all but two patients, heparin was given as a 5,000-U intravenous bolus at the end of the rt-PA infusion followed by a 1,000-U/h continuous infusion. In two patients, heparin was given before the start of the rt-PA infusion as a 5,000-U intravenous bolus followed by a 1,000-U/h intravenous infusion. Subsequently, the dosage of heparin was adjusted to maintain the activated partial thromboplastin time at 2 to 2.5 times that in control plasma. This study was approved by the Human Studies Committee at Washington University School of Medicine.

Acquisition of blood samples. Blood samples were obtained from participants on their admission to the cardiac care unit. They were obtained by venipuncture except during the rt-PA infusion, when they were drawn through a large bore heparin lock (18-gauge). Samples were obtained before initiation of the rt-PA infusion and 2, 3, 4 and 8 h after the start of the infusion. In some patients, additional samples were obtained at 1, 12 and 24 h after the start of rt-PA infusion. All samples were drawn by specially trained technicians and cooled immediately to 4°C in an ice bath for 20 min. Blood was drawn into collection tubes containing EDTA, aprotinin (200-KIU final concentration), and D-phenylalanyl-L prolyl-L arginine-chlorylmethylketone (PPACK) at a final concentration in excess of 5 μM to inhibit both rt-PA and thrombin. Platelet-poor plasma was prepared by centrifugation of each sample at 1,500 g for 30 min. The plasma was frozen at −20°C for <24 h and then at −70°C until it was assayed. The procedures used for sample acquisition and processing have been validated extensively in previous studies (1,10,15).

Assay of prothrombin fragment 1.2. The enzyme-linked immunosorbent assay (ELISA) for prothrombin fragment 1.2 employed a recently developed monoclonal antibody that is specific for an antigenic site on the prothrombin fragment 1.2 molecule absent in native prothrombin (16). The ELISA reagents were provided by Baxter Diagnostics, Inc. Plasma samples were incubated for 30 min in wells of a microtiter plate coated with the monoclonal antibody specific for prothrombin fragment 1.2. The plate was washed twice, and a second monoclonal antibody conjugated to horseradish peroxidase that also recognizes prothrombin fragment 1.2 was incubated on the plate for 10 min at room temperature. After washing, the horseradish peroxidase substrate, 3, 3', 5, 5' tetramethylbenzidine, was added and color was subsequently intensified by addition of 1 N sulfuric acid. The absorbance attributable to hydrolysis of the substrate was monitored at 450 nM in an automated microtiter plate reader (ThermoMax, Molecular Devices). The concentration of prothrombin fragment 1.2 in the plasma samples was determined by comparison with concurrently run standards of purified human prothrombin fragment 1.2. All samples were run in duplicate. The normal range for concentrations of prothrombin fragment 1.2 in plasma with this assay is 0.3 to 1.1 nM (16). In samples obtained from healthy young nonhospitalized volunteers and processed in our laboratory in a manner identical to that used in the current study, concentrations of prothrombin fragment 1.2 were found to be somewhat lower, 0.16 ± 0.08 nM.

Statistical analysis. Data are expressed as mean values ± SD. Because values for the concentrations of prothrombin fragment 1.2 were not distributed normally, a log transformation was used before data were analyzed statistically. Values for the log concentration of prothrombin fragment 1.2 were distributed normally after the transformation. Concentrations of prothrombin fragment 1.2 at each time point after initiation of rt-PA were compared with those on admission (before rt-PA) with a two-tailed paired t test. Differences were considered to be significant when the p values were < 0.05.

Results

Concentrations of prothrombin fragment 1.2 in plasma. The plasma concentrations were 0.89 ± 1.2 nM before initiation of the infusion of rt-PA in the 20 patients with acute myocardial infarction not given early heparin. In three patients, the concentrations exceeded 1.0 nM. In the two patients given heparin before rt-PA, the concentrations were

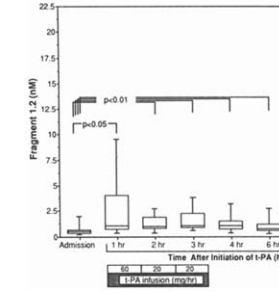

Figure 2. Concentration of prothrombin fragment 1.2 before and after initiation of rt-PA infusion in 22 patients with acute myocardial infarction. The **box plot** shows the range of values associated with 50% of the values (25th to 75th percentile) at each time point; the median concentration is indicated by the **line** in each box. The concentrations for the 10th and 90th percentiles are indicated by the **bars**. The concentration of prothrombin fragment 1.2 increased significantly over admission values after initiation of the rt-PA infusions. Smaller increases occurred during the 2nd and 3rd h of the rt-PA infusions. The concentrations of prothrombin fragment 1.2 decreased significantly after initiation of heparin infusion at the end of the rt-PA infusions. However, an increase in concentration of prothrombin fragment 1.2 compared with that at admission was noted despite treatment with heparin for up to 6 h after initiation of rt-PA.

0.15 and 0.28 n*M* after heparin but before rt-PA. Concentrations of prothrombin fragment 1.2 increased after initiation of rt-PA for the group as a whole to a mean of 1.5 ± 1.5 n*M* 2 h after onset of the rt-PA infusion ($p < 0.05$ compared with admission values) (Fig. 2). Concentration of prothrombin fragment 1.2 increased over pre-rt-PA values in all but 4 patients; in 10 patients concentrations of prothrombin fragment 1.2 increased twofold. More marked increases occurred within the 1st h after the initiation of rt-PA infusion in the 12 patients whose samples were available at that time point (3.4 ± 5.5 n*M*). In the two patients given a 5,000-U bolus of heparin before rt-PA followed by a continuous heparin infusion of 1,000 U/h, the concentration of prothrombin fragment 1.2 increased only minimally from 0.15 and 0.28 n*M* after heparin but before rt-PA, to 0.3 and 0.39 n*M* 2 h after rt-PA.

Response to heparin. Twenty patients were given a 5,000-U intravenous bolus of heparin at the end of the rt-PA infusion, followed by a continuous heparin infusion. In the other two patients, heparin was given before rt-PA. The concentration of prothrombin fragment 1.2 decreased significantly in the patients who were given heparin after rt-PA, from 1.8 ± 1.8 n*M* before heparin to 1.1 ± 0.87 n*M* 1 h after initiation of heparin ($p = 0.02$). However, the concentration of prothrombin fragment 1.2 remained elevated compared with baseline values (1.3 ± 1.1 n*M*), with values >1.2 n*M* in 10 patients while heparin was being infused at a time when the activated partial thromboplastin time was greater than twice control in all but one patient.

Discussion

Our results indicate that activation of prothrombin occurs in patients with acute myocardial infarction treated with rt-PA, which appears to account at least in part for the increased thrombin activity that has been documented to accompany pharmacologic thrombolysis (1,4,10). In previous studies (15) we have shown that thrombin activity, as reflected by changes in the concentrations of fibrinopeptide A, does not increase in the first few hours after the onset of acute myocardial infarction when patients are not treated with fibrinolytic agents. Thus, the results of the current study are consistent with the induction of procoagulant activity by fibrinolytic agents. Our results suggest that conjunctive anticoagulant agents given to inhibit activation of prothrombin may be of particular value in this setting and underscore the importance of concomitant anticoagulation in patients treated with rt-PA.

Mechanisms for activation of prothrombin during pharmacologic thrombolysis. One potential mechanism underlying the activation of prothrombin accompanying thrombolysis is activation of factor V by plasmin, as shown by Lee and Mann (17). Because activation of prothrombin by factor Xa is markedly accelerated by factor Va, increased concentrations of Va induced by plasmin proteolysis could account for an increased prothrombin activation (18). Increased activation of prothrombin suggests that the activity of the factor Xa/Va complex may be an important determinant of the extent of thrombin activity induced by fibrinolytic agents. Thus, inhibition of this complex may be an ideal target for conjunctive anticoagulation in patients treated with fibrinolytic agents. The recent observation (19) that tick anticoagulant protein, a specific inhibitor of factor Xa, prevents reocclusion in experimental preparations of coronary thrombolysis is consistent with this hypothesis. Because the Xa/Va complex can activate many prothrombin molecules, its inhibition may be more efficient than inhibition of thrombin in attenuating procoagulant effects accompanying thrombolysis.

Role of heparin in inhibiting activation of prothrombin during thrombolysis. The extent to which procoagulant activity increased in response to rt-PA in our study was probably influenced by the decision to initiate heparin at the end of the rt-PA infusion in most patients. In the two patients given heparin before rt-PA, increases in the concentration of prothrombin fragment 1.2 were considerably less marked. The lack of increase in prothrombin fragment 1.2 in these two patients suggests that heparin may prevent the activation of prothrombin induced by administration of rt-PA. These results are consistent with the results of previous studies (4,5) in which increased plasma concentrations of

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fibrinopeptide A and thrombin-antithrombin III complexes accompanying treatment with rt-PA were found to be attenuated after initiation of intravenous heparin. Although the results of the TAMI-3 study (20) suggest that delaying the initiation of heparin does not decrease the incidence of patency of the infarct-related artery at 90 min after the onset of treatment with rt-PA, increases in procoagulant activity could adversely affect the persistence of patency. In addition, early increases in procoagulant activity could delay clot lysis (even within the 90-min TAMI-3 observation period) or account, in part, for the lower incidence of patency evident 1 to several days later in patients treated with rt-PA without heparin (2,3). The importance of inhibition of thrombin is evident from recent results (21) in a dog model of coronary thrombosis in which conjunctive treatment with hirudin, a potent antithrombin, accelerated coronary thrombolysis and prevented reocclusion. One limitation in interpretation of results of the present study is the possibility that the decreases in the concentration of prothrombin fragment 1.2 we observed after initiation of heparin therapy, although statistically significant, would have occurred even in the absence of heparin.

Value of monitoring procoagulant activity in patients treated with fibrinolytic agents. Although our study was not specifically designed to characterize the utility of monitoring patients for procoagulant activity during thrombolytic therapy, our results suggest that assay of prothrombin fragment 1.2 may provide useful clinical information. Other studies (1,4) have shown greater increases in the concentration of fibrinopeptide A, a marker of thrombin activity, in patients in whom reocclusion occurs after treatment with rt-PA or streptokinase. Increases in the concentration of thrombin-antithrombin III complexes, a measure of elaboration of thrombin, also appear to identify patients in whom reperfusion does not occur and those prone to reocclusion as well (5). Bauer and Rosenberg (22) found that prothrombin fragment 1.2 is more sensitive than fibrinopeptide A as a marker of thrombosis in patients with thrombotic disorders. Whether changes in the concentration of prothrombin fragment 1.2 will be of prognostic value in patients treated with fibrinolytic agents for myocardial infarction is not yet clear, but the possibility appears likely. Increases in the concentration of prothrombin fragment 1.2 in plasma can occur with very high concentrations of plasmin in plasma as a direct result of proteolysis of prothrombin by plasmin (23,24). In addition, the specificity of antibodies used by others for prothrombin fragment 1.2 may influence results and lead to differences with respect to our observations. Nonetheless, characterization of activation of prothrombin accompanying thrombolysis may be of value clinically, particularly if anticoagulant agents designed to inhibit Xa/Va complex activity prove to be useful as conjunctive agents.

Clinical implications. The finding that prothrombin activation accompanies thrombolysis when heparin is not given suggests that the increases in thrombin activity observed previously are at least in part attributable to increased elaboration of thrombin rather than simply the activity of preformed thrombin associated with clots. Because activation of prothrombin reflects activity of the factor Xa/Va complex, one implication of our data is that inhibition of earlier steps in the coagulation cascade may be a useful target for conjunctive anticoagulation in the setting of thrombolysis.

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