Recombinant t-PA and platelet activity

RICHARD C. BECKER, MD

■ Platelet-rich coronary arterial occlusions are relatively resistant to thrombolysis with recombinant tissue-type plasminogen activator, or rt-PA, while erythrocyte-rich thrombi are lysed at low doses. The combined administration of rt-PA with a platelet antagonist enhances thrombolysis and can prevent reocclusion, which suggests that platelet activation predominates during thrombus formation and after thrombolysis. In addition to direct effects on platelet activity, rt-PA also acts indirectly through generation of plasmin, which degrades fibrin.

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RECOMBINANT tissue-type plasminogen activator (rt-PA) is a fibrin-specific thrombolytic agent used frequently in the treatment of acute myocardial infarction. There is firm evidence that rt-PA lyses intracoronary thrombi, reduces infarct size, preserves left ventricular function and improves patient survival. Its primary mechanism of action involves the generation of plasmin which subsequently degrades fibrin, but rt-PA has direct and indirect effects on platelet structure and function as well. The importance of this interaction should not be underestimated, since the effects of rt-PA on platelet activity could have a direct impact on thrombolytic efficacy, coronary arterial reocclusion, and hemorrhagic risk.

MOLECULAR STRUCTURE OF T-PA

Tissue-type plasminogen activator (t-PA) is a serine protease that catalyzes the conversion of plasminogen to plasmin (*Figure 1*). It has a molecular weight of 78 KDa and has been isolated from a variety of mammalian tissues; however, the principal site of synthesis is the endothelial cell.[1]

The complete DNA sequence of t-PA has been determined and contains a single open reading frame, beginning with the ATG codon at nucleotides 85 to 87, which is followed 562 codons later by a TGA termination triplet at nucleotides 1771 to 1773.[2] Single-chain t-PA is converted easily to a double-chain form by plasmin through cleavage of the peptide bond Arg-278-Ile-279. The enzymatic activity of t-PA is enhanced nearly 1,000-fold in the presence of fibrin, which reflects the increased affinity of fibrin-bound plasminogen for t-PA. Both t-PA and plasminogen adsorb to fibrin in a sequential and orderly fashion to yield a ternary complex; this, in turn, increases the generation of plasmin at the site of thrombus formation.[3,4] However, the specificity of t-PA–induced plasminogen activation is relative rather than absolute; some activation of the systemic fibrinolytic pathway does occur at high concentrations of serum t-PA antigen.

Primary sequence analysis has allowed the identification of specific domains within the t-PA molecule. These include a fibronectin finger domain, an epidermal growth factor domain, two kringle domains, and a serine protease domain (*Figure 2*).[5]

From the Thrombosis Research Center, Division of Cardiology, University of Massachusetts Medical Center, Worcester.

Address reprint requests to R.C.B., associate director, Coronary Care Unit, director, Thrombosis Research Center, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, Massachusetts 01655.

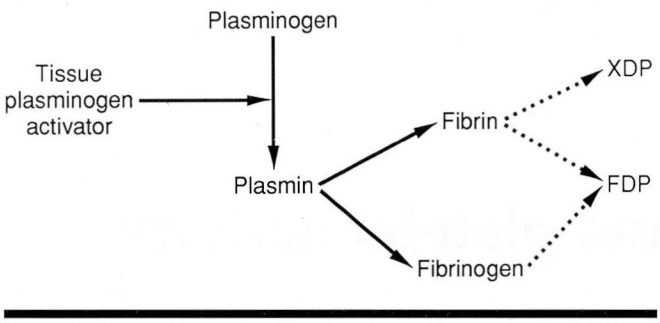

FIGURE 1. Tissue plasminogen activator converts plasminogen to the catalytically active, serine protease plasmin. FDP, fibrin(ogen) degradation products; XDP, cross-linked fibrin degradation products.

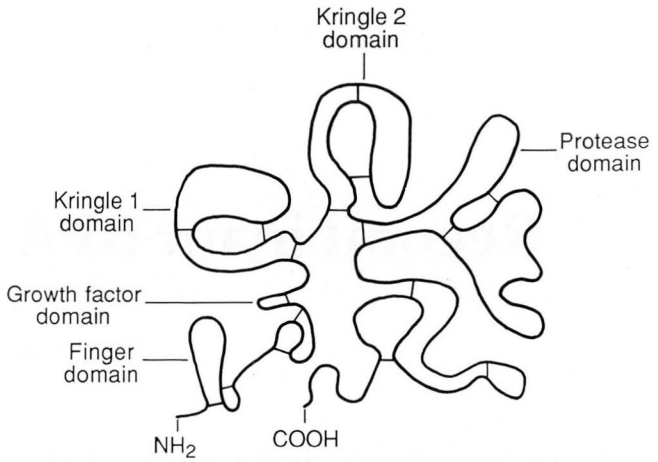

FIGURE 2. Molecular structure of tissue-type plasminogen activator identified by primary sequence analysis.

Currently, the single-chain form of t-PA is manufactured for clinical use by means of recombinant DNA technology (rt-PA) as alteplase (Activase). The double-chain form is investigational.

PLATELET PHYSIOLOGY

The primary functions of platelets in the hemostatic mechanism include adhesion to damaged vessel walls, aggregation to form a platelet plug, and promotion of fibrin clot formation. Platelet adhesion is mediated by certain platelet surface receptors with high affinity for subendothelial adhesive glycoproteins. Several of the receptors (GPIa/IIa, GPIc/IIc, GPIIb/IIIa) belong to the integrin family of receptors found on a variety of cells. Certain other receptors such as GPIb (the von Willebrand factor receptor) are also important. Following stimulation, platelet membrane phospholipases are activated which liberate arachidonic acid for subsequent conversion to unstable prostaglandin intermediates (PGG_2 and PGH_2) by cyclooxygenase. PGH_2 is then metabolized to the potent platelet aggregator thromboxane A_2.

In contrast to adhesion, which involves a number of surface receptors, platelet aggregation is mediated exclusively by the GPIIb/IIIa receptor. Thrombin is formed by activation of the intrinsic and extrinsic coagulation pathways. Thrombin then converts fibrinogen to fibrin which, when stabilized with Factor XIIIa, forms a cohesive clot.

PLATELET- V ERYTHROCYTE-RICH THROMBI

Coronary artery thrombi consist of both platelet-rich and erythrocyte-rich zones, with the former predominating in most instances. Platelet-rich zones exist primarily at the site of atherosclerotic plaque rupture, while erythrocyte-rich zones develop both proximal and distal to the area of vascular injury (*Figure 3*).[6]

Under ideal conditions, coronary artery reperfusion following thrombolytic therapy with rt-PA is achieved in only 75% to 80% of patients. Therefore, with current dosing regimens, 20% to 25% of all patients with acute coronary arterial occlusion appear to be resistant to thrombolysis. While thrombolytic resistance may result from nonthrombotic mechanisms of occlusion such as coronary arterial vasospasm, luminal compression by intraplaque hemorrhage, and arterial dissection, intrinsic resistance of the thrombus to lysis must also be considered.

Preformed whole blood or plasma clots are lysed readily with low concentrations of rt-PA, whereas platelet aggregates formed in platelet-rich plasma require a much higher concentration of rt-PA for disaggregation.[7] Similarly, erythrocyte-rich coronary arterial thrombi are consistently lysed following an intravenous infusion of rt-PA at low doses. In contrast, platelet-rich thrombi are relatively resistant to lysis even at high doses.[8]

Adjuvant pharmacologic therapy combining rt-PA and antiplatelet agents (thromboxane receptor antagonists, serotonin receptor antagonists, and monoclonal GPIIb/IIIa antibodies) have yielded encouraging results suggesting that thrombolytic resistance, at least in some settings, may be mediated by enhanced platelet activity.[8–11] In addition, platelets may play a central role in coronary reocclusion following initially successful thrombolysis.[12,13]

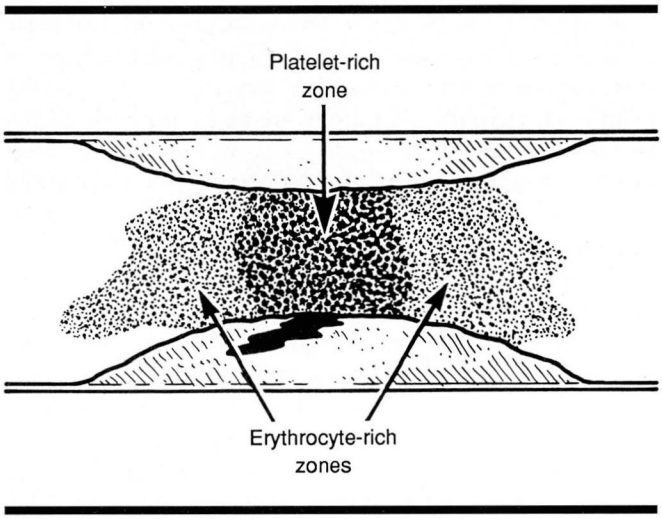

FIGURE 3. Schematic representation of a coronary arterial thrombus with a platelet-rich zone at the site of plaque rupture and erythrocyte-rich zones extending both proximally and distally.

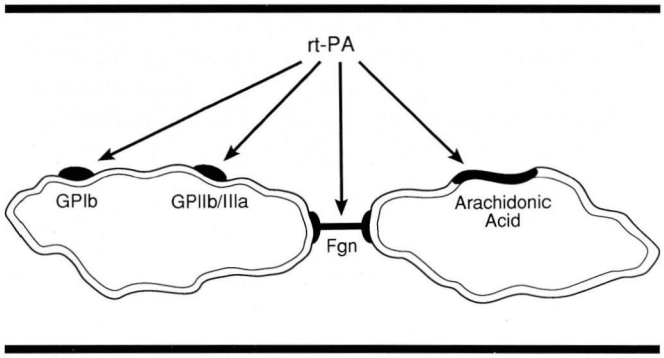

FIGURE 4. Schematic representation of the mechanisms responsible for rt-PA's antiplatelet effect. rt-PA (or plasmin) results in proteolysis of GPIb, GPIIb/IIIa, and fibrinogen bridges, and prevents arachidonic acid mobilization from the platelet surface membrane. GPIb, glycoprotein Ib receptor; GPIIb/IIIa, glycoprotein IIb/IIIa complex receptor; Fgn, fibrinogen bridge.

T-PA AND PLATELET INTERACTIONS

The effects of t-PA on platelet surface receptor glycoproteins, specifically glycoprotein Ib (GPIb), have been examined previously in patients participating in the Thrombolysis and Myocardial Infarction (TIMI) trial—Phase II.[14] Following an intravenous infusion of rt-PA, an increase in plasma glycocalicin (a proteolytic fragment derived from GPIb) was noted. In addition, a concomitant decrease in whole platelet GPIb and a slight increase in platelet surface GPIb was observed, suggesting that platelets can replenish their surface GPIb over time by recruitment of molecules from an intraplatelet storage pool.[14] The clinical relevance of the observed changes in GPIb following rt-PA administration is being explored currently in a larger patient cohort.

Platelet aggregates in suspension undergo disaggregation following incubation with rt-PA. Aspirin potentiates this effect and alpha$_2$ plasmin inhibitor (α_2 PI) attenuates it. Although the mechanisms are still being explored, comparative analyses of the rate of platelet-bound fibrinogen proteolysis with ambient plasma fibrinogen suggest that rt-PA facilitates platelet disaggregation through kinetically selective proteolysis of cohesive fibrinogen.[7] In addition, rt-PA may affect intracellular signalling; as a result, it may attenuate calcium rise and serotonin secretion,[15] thereby impairing platelet aggregation.

In vitro inhibition of platelet activation occurs in saline as well as in plasma suspensions and thus does not appear to be dependent on plasma factors.[16] Moreover, rt-PA may derive antiplatelet activity through endothelium-derived relaxing factor-like properties.[17]

In concentrations commonly achieved after a standard intravenous bolus[18] or during a maintenance infusion[19,20] of rt-PA, platelet aggregation in response to a wide variety of stimuli (including ristocetin, adenosine diphosphate [ADP], thrombin, and collagen) is attenuated, suggesting that GPIIb/IIIa (the fibrinogen receptor) may also be affected.

Although it has direct effects on platelet, rt-PA also acts through indirect means via plasmin generation. The overall effect of plasmin on platelet function appears to be concentration-dependent. At low concentrations (less than 0.5 CU/ml), plasmin inhibits platelet aggregation in response to thrombin, ionophore A23187, and collagen. It appears that plasmin inhibits platelet function, at least in part, by blocking the mobilization of arachidonic acid from surface membrane phospholipid pools (*Figure 4*).[21] Plasmin also exerts antiplatelet effects through the generation of fibrin(ogen) degradation products.[22]

In contrast, higher concentrations of plasmin (greater than 1.0 CU/ml) have been shown to increase platelet aggregation. A number of mechanisms have been proposed: (1) plasmin-treated platelets have an increased number of available fibrinogen-binding sites on their surface[23]; (2) platelet suspensions incubated with plasmin undergo activation, and release ADP, serotonin, and fibrinogen from internal storage granules[24]; and (3)

plasmin mediates a time- and dose-dependent phosphorylation of platelet proteins, causing a rise in the concentration of cytosolic calcium and inositol phospholipid-dependent phospholipase C, and an increase in protein kinase C activation.[25]

COMPLICATIONS

The major complication associated with the administration of rt-PA, and with other fibrinolytic agents as well, is hemorrhage. Although bleeding is most common at sites of vascular trauma, spontaneous bleeding may also occur.

Despite the fibrin specificity of rt-PA, hemorrhagic complications associated with its use have not differed substantially from those observed with other fibrinolytic agents, suggesting a role for factors other than, or in addition to, systemic fibrinogenolysis.

The ability to predict patients at highest risk for spontaneous bleeding has been problematic. Comparative controlled studies have shown a relatively weak correlation between the extent of fibrinogen degradation and bleeding tendency[20,26]; moreover, extreme interindividual variability has prevented the use of fibrinolytic and hemostatic serum markers in a clinically relevant fashion.[27]

Several recent observations suggest that rt-PA- or plasmin-mediated effects on platelet function may play a central role in the pathogenesis of hemorrhagic complications. In a study of 52 patients with acute myocardial infarction, Gimple and associates observed a significant increase in template bleeding time 90 minutes into the rt-PA infusion.[28] Bleeding times were more prolonged in those taking aspirin as compared with patients not taking aspirin. In a multivariate analysis, only the 90-minute bleeding time correlated with spontaneous bleeding ($P = 0.01$).

Vaughan and colleagues examined the effects of aspirin and rt-PA on serial template bleeding times and bleeding tendency in rabbits.[29] The intravenous administration of aspirin prolonged the bleeding time more than rt-PA; however, the most profound increase in bleeding time was observed when a combination of intravenous aspirin and rt-PA was given ($P < 0.01$). Moreover, virtually all animals treated with both agents manifested a bleeding tendency, as evidenced by spontaneous rebleeding at sites of previously performed bleeding times or bleeding at femoral venous catheterization sites.

Of potential clinical relevance, an intravenous bolus injection of recombinant human plasminogen activator inhibitor-1 (rPAI-1) resulted in complete reversal of the prolongation of bleeding time and in the neutralization of the bleeding tendency.[27] The ability of rPAI-1 to correct the bleeding tendency associated with rt-PA suggests that it may be of value as a specific "antidote" for bleeding complications. Further investigation of rPAI-1 is warranted, however, before specific recommendations for its use can be made.

CONCLUSION

The effects of rt-PA on platelet activity are complex. The ability to enhance coronary arterial thrombolysis and prevent vessel reocclusion with the combined administration of rt-PA and a platelet antagonist (thromboxane A_2 receptor antagonist, serotonin receptor antagonist, monoclonal GPIIb/IIIa antibody) suggests that platelet activation predominates at the site of vascular injury both during thrombus formation and following thrombolysis.[8–13] However, the effects observed peripherally may differ substantially from those occurring focally, thereby explaining, at least in part, the paradox of concomitant coronary arterial thrombogenesis and systemic bleeding tendency. There is encouraging evidence that platelet activity, and the effects of thrombolytic therapy thereon, may hold the key to our understanding of acute myocardial infarction, as well as its safe and effective treatment.

REFERENCES

1. Levin EG, Loskutoff DJ. Cultured bovine endothelial cells produce both urokinase and tissue-type plasminogen activators. J Cell Biol 1982; **94**:631–636.
2. Pennica D, Holmes WE, Kohr WJ, et al. Cloning and expression of human tissue-type plasminogen activator cDNA in E. coli. Nature 1983; **301**:214–221.
3. Bachmann F, Kruithof EKO. Tissue plasminogen activator: chemical and physiological aspects. Semin Thromb Hemost 1984; **10**:6–17.
4. Wallen P, Bergsdorf N, Ranby M. Purification and identification of two structural variants of porcine tissue plasminogen activator by affinity adsorption on fibrin. Biochem Biophys Acta 1982; **719**:318–328.
5. Ny T, Elgh F, Lund B. The structure of human tissue-type plasminogen activator gene: correlation of intron and exon structures to functional and structural domains. Proc Natl Acad Sci USA 1984; **81**:5355–5359.
6. Friedman M, Van den Bovenkamp EJ. The pathogenesis of a coronary thrombus. Am J Path 1966; **48**:19–44
7. Loscalzo J, Vaughan DE. Tissue plasminogen activator promotes platelet disaggregation in plasma. J Clin Invest 1987; **79**:1749–1755.
8. Jang IK, Gold HK, Ziskind AA, et al. Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator: a possible explanation for resistance to coronary thrombolysis. Circulation 1989; **79**:920–928.
9. Golino P, Ashton JH, Glas-Greenwalt P, McNatt J, Buja LM, Willerson JT. Mediation of reocclusion by thromboxane A_2 and serotonin after thrombolysis with tissue-type plasminogen activator in a canine preparation of coronary thrombosis. Circulation 1988; **77**:678–684.
10. Golino P, Ashton JH, McNatt J, et al. Simultaneous administration of thromboxane A_2 and serotonin receptor antagonists markedly enhance thrombolysis and prevent or delay reocclusion after tissue-type plas-

minogen activator in a canine model of coronary thrombosis. Circulation 1989; **79:**911–919.
11. Gold HK, Coller BS, Yasuda T, et al. Rapid and sustained coronary artery recanalization with combined bolus injection of recombinant tissue-type plasminogen activator and monoclonal antiplatelet GPIIb/IIIa antibody in a canine preparation. Circulation 1988; **77:**670–677.
12. Haskel EJ, Adams SP, Feigen LP, et al. Prevention of reoccluding platelet-rich thrombi in canine femoral arteries with a novel peptide antagonist of platelet glycoprotein IIb/IIIa receptors. Circulation 1989; **80:**1775–1782.
13. Mickelson JK, Simpson PJ, Cronin M, et al. Antiplatelet antibody [7E3 $FAb')_2$] prevents rethrombosis after recombinant tissue-type plasminogen activator-induced coronary artery thrombolysis in a canine model. Circulation 1990; **81:**617–627.
14. Michaelson AD, Barnard MR, Rybak ME, Gore JM, Cola CA. In vivo infusion of recombinant tissue-type plasminogen activator results in a redistribution of platelet glycoprotein Ib stores. Circulation 1988; **78(Suppl II):** II-620.
15. Penney WF, Ware JA. Platelet inhibition *in vitro* by tissue plasminogen activator and streptokinase. Circulation 1989; **80(Suppl II)** II-218.
16. Terres W, Umnus-Schnelle S, Hamm CW, Bleifeld W. In vitro effects of thrombolytic agents on platelets. J Am Coll Cardiol 1990; **15(Suppl A):**189A.
17. Yang Z, von Segesser L, Bauer E, Turina M, Luscher TF. Tissue-type plasminogen activator relaxes human coronary and mammary arteries: a new endothelium-derived relaxing factor? J Am Coll Cardiol 1990; **15(Suppl A):**180A.
18. Grace AM, Fry ETA, Sobel BE. Decreased aggregation of platelets induced by exposure to Pharmacologic concentrations of t-PA in whole blood. Circulation 1987; **76(Suppl IV):**IV-307.
19. Stricker RB, Wong D, Tak Shiu D, Reyes PT, Shuman MA. Activation of plasminogen by tissue plasminogen activator on normal and thrombasthenic platelets; effects on surface proteins and platelet aggregation. Blood 1986; **68:**275–280.
20. Rao AK, Pratt C, Berke A, et al for the TIMI Investigators. Thrombolysis in Myocardial Infarction (TIMI) Trial—Phase I: hemorrhagic manifestations and changes in plasma fibrinogen and the fibrinolytic systems in patients treated with recombinant tissue plasminogen activator and streptokinase. J Am Coll Cardiol 1988; **11:**1–11.
21. Schafer AI, Adelman B. Plasmin inhibition of platelet function and of arachidonic acid metabolism. J Clin Invest 1985; **75:**456–461.
22. Kowalski E. Fibrinogen derivatives and their biologic activities. Semin Hematol 1968; **5:**45–59.
23. Greenberg JP, Packham MA, Guccione MA, et al. The effect of pretreatment of human or rabbit platelets with chymotrypsin on their responses to human fibrinogen and aggregating agents. Blood 1979; **54:**753–765.
24. Guccione MA, Kinlough-Rathbone RL, Packham MA, et al. Effects of plasmin on rabbit platelets. Thromb Hemost 1985; **53:**8–14.
25. Schafer AI, Maas AK, Ware JA, Johnson PC, Rittenhouse SE, Salzman EW. Platelet protein phosphorylation, elevation of cytosolic calcium, and inositol phospholipid breakdown in platelet activation induced by plasmin. J Clin Invest 1986; **78:**73–79.
26. Arnold AER, Brower RW, Collen D, et al for the European Cooperative Study group for rt-PA. Increased serum levels of fibrinogen degradation products due to treatment with recombinant tissue-type plasminogen activator for acute myocardial infarction are related to bleeding complications, but not to coronary patency. J Am Coll Cardiol 1989; **14:**581–588.
27. Stump DC, Califf RM, Topol EJ, et al, and the TAMI Study group. Pharmacodynamics of thrombolysis with recombinant tissue-type plasminogen activator: correlation with characteristics of and clinical outcomes in patients with acute myocardial infarction. Circulation 1989; **80:**1222–1230.
28. Gimple LW, Gold HK, Leinbach RC, et al. Correlation between template bleeding times and spontaneous bleeding during treatment of acute myocardial infarction with recombinant tissue-type plasminogen activator. Circulation 1989; **80:**581–588.
29. Vaughan DE, Declerck PJ, De Mol M, Collen D. Recombinant plasminogen activator inhibitor-1 reverses the bleeding tendency associated with the combined administration of tissue-type plasminogen activator and aspirin in rabbits. J Clin Invest 1989; **84:**586–591.