

Tissue-Type Plasminogen Activator Mutants
Theoretical and Clinical Considerations

Nils U. Bang, MD

Within a short time after the first report on the successful cloning and expression of tissue-type plasminogen activator (t-PA),1 several laboratories began working on creating new t-PA derivatives through site-directed mutagenesis.2-4 t-PA has unique properties that make it an attractive choice for thrombolytic therapy. t-PA is “fibrin directed”; that is, it preferentially activates the fibrinolytic proenzyme plasminogen into plasmin on fibrin thrombi. In contrast, streptokinase and urokinase, the older-used thrombolytic agents, indiscriminately activate thrombus as well as plasma plasminogen.3 Nevertheless, work on creating new t-PA mutants continued because the following shortcomings of the native enzyme were realized: 1) t-PA is not a very efficient plasminogen activator even on a fibrin thrombus; 2) although t-PA was anticipated to cause minimal bleeding complications, this prediction was not confirmed in major clinical trials in which t-PA was given in the doses necessary for effective coronary thrombolysis (100 mg per treatment); it proved as likely to cause minor and major bleeding complications as streptokinase and in some patients caused profound fibrinogen depletion and a serious coagulation defect6-8; 3) t-PA, even when given concomitantly with or followed by heparin, carried a high risk of reocclusion (10-20%) once reperfusion has been established; 4) the activity of infused t-PA is attenuated because it is neutralized by a number of plasma protease inhibitors, most notably plasminogen activator inhibitor 1 (PAI-1); and 5) t-PA possesses a very short biologic half-life (<5 minutes) in humans and, therefore, must be administered in high doses as a constant intravenous infusion. The study by C.E.L. Lucore, S. Fuji and activity, fibrin affinity, and interaction with inhibitors, specifically PAI-1.

t-PA probably arose through exon shuffling, and specific domains encoded by specific exons in t-PA harbor specific and independent functions.2 The major domains are called “finger” (F), epidermal growth factor (EGF), kringle 1 (K1), kringle 2 (K2), and serine protease (SP). The F and K2 domains have been shown by several laboratories to be involved in t-PA binding to fibrin.2 The EGF domain may play a role in t-PA binding to cells. The K1 domain has not been assigned a function with certainty except that it contains on residue Asn 117, a complex, high mannose branched carbohydrate side chain that probably is responsible for the effective binding, internalization, and catabolism of t-PA by normal hepatocytes. The SP domain catalyzes the conversion of plasminogen into plasmin.5 The t-PA mutant examined by Lucore et al10 contains the F domain, a duplicated K2 domain, and the SP domain, but the mutant lacks the EGF and K1 domains. The lack of the EGF and K1 domains explains why this and similar molecules reported elsewhere possess prolonged biologic half-lives.3 Lucore et al10 elegantly show that the induction and maintenance of a thrombolytic state, whether the wild-type or mutant t-PA is used, depends entirely on the concentration of free plasminogen activator in the circulation. Thus, if the concentrations of inhibitors (e.g., PAI-1) are elevated, the fibrinolytic response to t-PA is “attenuated”; that is, active t-PA will remain in the circulation for a shorter time. On the other hand, if a t-PA mutant is introduced with substantially reduced clearance by hepatocytes and other cells during constant inhibitor levels, the mutant will circulate at higher levels and for longer time periods than the wild-type enzyme. The interesting question of whether t-PA mutants with prolonged t1/2 will result in therapeutic gains in acute myocardial infarction cannot be answered completely today. The mutant described by Lucore et al10 produced high enzyme levels for more prolonged time periods than did native t-PA. In theory, if the patient is treated early enough, reperfusion should be more rapid and additional salvage of ischemic myocardium should occur. There is also a distinct possibility that the incidence of reocclusion could be reduced by this approach. Prolonged admin-


B.E. Sobel10 in this issue of Circulation presents pharmacodynamic data on t-PA and an interesting t-PA mutant. The mutant has a sharpened t1/2 (30 minutes vs. 3 minutes for t-PA in the rabbit); in other respects, it is comparable to t-PA in specific

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From the Lilly Laboratories for Clinical Research, Eli Lilly and Company; and Indiana University School of Medicine, Departments of Medicine and Pathology, Wishard Memorial Hospital, 1001 W. 10th Street, Indianapolis, IN 46202.

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istration of t-PA after successful reperfusion has been reported to sharply lower the incidence of reocclusion. However, the prolonged administration of t-PA also resulted in a substantial increase in bleeding complications. Only prospective clinical trials can resolve whether t-PA mutants of prolonged t1/2 such as that described by Lucore et al can significantly increase the benefit to risk ratio of thrombolytic therapy in acute myocardial infarction. A separate issue is whether a plasminogen activator with a prolonged t1/2 in some instances can be detrimental to the patient if and when bypass surgery is contemplated in acute myocardial infarction.

Attempts to improve the functional properties of t-PA through site-directed mutagenesis have been less successful in other areas. Despite intensive efforts, the creation of chimeric proteins with the catalytic efficiency of urokinase and fibrin specificity of t-PA have not met with major success. Although increased fibrin specificity has been shown for certain mutants under in vitro conditions, the gains in this area have not been impressive, and whether such mutants will prove clinically superior to t-PA is uncertain. Although certain mutants with decreased affinity for the PAI-1 inhibitor can be constructed, the manipulations involved also result in loss in fibrin specificity and instability of the protein.

It is doubtful whether additional progress is going to be made with the current approach of removing or substituting entire domains in the t-PA protein. A complete understanding of the three-dimensional structure of t-PA and its domains appears to be the key to the construction of mutant proteins in which discrete point mutations lead to improved functional properties without serious changes in the conformation of the entire molecule.

Ancillary therapy may also be of chief importance for improved results of enzymatic intervention in acute myocardial infarction. Many investigators suspect that the combinations recommended today, for example, t-PA or streptokinase and heparin and/or aspirin, are suboptimal. Other candidate drugs more efficacious than heparin or aspirin on the arterial side of the circulation are activated protein C and antiplatelet agents, for example, thromboxane A2 receptor inhibitors in combination with 5-HT1 receptor antagonists, monoclonal antibodies, or fibrinogenomimetic peptides capable of blocking the important step in platelet aggregation (the binding of fibrinogen to the GPIIb/IIIa platelet receptor), and specific antithrombins such as hirudin, and a variety of synthetic inhibitors. Such approaches could improve our record in three crucial areas of acute intervention in acute myocardial infarction: time to reperfusion, reduction of reocclusion events, and reduction of bleeding complications.

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N U Bang

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