Thrombosis of a coronary or cerebral artery is the most common cause of mortality and morbidity in the developed nations. The proximate cause is most often atherosclerosis, and the most appropriate intervention is its prevention. Much progress has been made in identifying the risk factors predisposing to atherosclerosis, and, perhaps because of their recognition, a significant decrease in the incidence of both stroke and myocardial infarction has occurred. However, coronary thrombosis remains a major clinical problem. Early dissolution of the thrombus by plasminogen activator therapy has become the most effective means of limiting myocardial damage.

With the Food and Drug Administration's recent approval of tissue plasminogen activator (t-PA), the clinician now has available several agents for the treatment of acute coronary thrombosis. Three plasminogen activators are currently available in the United States: urokinase, streptokinase, and t-PA (the last two are FDA approved). Two more agents are on the horizon: acylated plasminogen-streptokinase activator complex (APSAC) and single-chain urokinase plasminogen activator (scu-PA). The economics and logistics of choosing a plasminogen activator have been discussed recently in detail. However, the information necessary for the physician to be able to choose among the various plasminogen activators—a direct clinical comparison—is lacking. Two large-scale clinical trials now underway, GISSI-II (Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Mio- cardico) and ISIS-III (International Studies of Infarct Survival), will compare t-PA and streptokinase and will evaluate the concomitant use of anticoagulant and antiplatelet agents. Therefore, in this review, we do not recommend the use of a particular plasminogen activator, but we focus instead on the theoretical and practical considerations for the current development of new agents and discuss properties that would be desirable for the ideal plasminogen activator of the future.

The surge in interest in the clinical use of plasminogen activators coincides with a new ability to use the tools of molecular biology to understand and modify them. For nearly a decade, the focus of investigation has been on the development of plasminogen activators that could be targeted to the fibrin in a blood clot. Now on the horizon are agents with markedly increased fibrin selectivity and other clinically important modifications such as increased half-life and resistance to inactivation in plasma. Other targets for more selective thrombolysis may well be identified, leading to the development of agents with yet greater specificity and safety.

We begin this review by discussing the basic aspects of thrombolysis, the role of plasminogen in thrombolysis, and the structure and function of the plasminogen activators. We then describe the pharmacologic effects of plasminogen activators, summarize the clinical data on plasminogen activator therapy for acute myocardial infarction, and examine what conclusions can be drawn from the available clinical studies of streptokinase, t-PA, and APSAC. Because a complete understanding of the potential advantages of new plasminogen activators requires a knowledge of their structural and biochemical properties, we also discuss how an understanding of these properties has allowed modifications of available molecules. Finally, we turn our attention to the fibrin selectivity of plasminogen activators as a model of an intrinsic property of certain naturally occurring proteins and as a property that can be enhanced through the methods of protein engineering.

Basic Aspects of Thrombolysis and Role of Plasminogen

The enzyme pivotal to fibrinolysis in humans is plasmin. Plasmin is a fully active serine protease formed by the action of plasminogen activators on its zymogen precursor, plasminogen. Although plasmin's favored substrate is fibrin (which forms the structural framework of thrombi), plasmin is not a very selective enzyme. It also degrades circulating fibrinogen, clotting factors V and VIII, and platelet membrane receptors (thereby altering platelet function).
Plasminogen can be found both free in the circulation and bound to fibrin. The conversion of substantial amounts of free plasminogen to plasmin is thought to cause the bleeding complications that accompany plasminogen activator therapy. Normally, there is very little active plasmin in the circulation, and whatever plasmin is present is inhibited by α2-antiplasmin.

Streptokinase and urokinase activate plasminogen both at the clot surface and in the plasma, which causes the release of more plasmin than can be inhibited by available α2-antiplasmin. Unchecked, the systemic release of plasmin is capable of paralyzing the clotting process by destroying many of its components, causing a “lytic state.”

**Structure and Function of Plasminogen Activators**

First-generation plasminogen activators (streptokinase and urokinase) are compounds found in nature that lack fibrin selectivity. Second-generation activators (t-PA and scu-PA) are natural human proteins that possess some fibrin selectivity and can be produced either by selected human cell cultures or by recombinant DNA methods. Third-generation plasminogen activators (APSAC and numerous compounds in early development) are natural proteins that possess enhanced properties as a result either of chemical modification or of the application of recombinant DNA technology.

Streptokinase, a bacterial protein, and urokinase, a human enzyme initially purified from urine, were the first plasminogen activators to be used clinically. Each has a very different mechanism of action. Urokinase, an active enzyme, directly cleaves a specific peptide bond in plasminogen and thereby converts it into plasmin. Streptokinase, on the other hand, is not an enzyme and cannot by itself cleave this peptide bond. To activate plasminogen, streptokinase must first bind to it in a 1:1 stoichiometric ratio. This binding changes the molecular conformation of plasminogen so that it becomes an active enzyme without peptide bond cleavage. The plasminogen-streptokinase complex then cleaves other plasminogen molecules to activate them. Because neither streptokinase nor urokinase binds directly to fibrin, these plasminogen activators are as active in plasma as they are on the clot.

t-PA and scu-PA, the second generation of plasminogen activators, are human proteins secreted by a variety of tissues, including the endothelium. Small quantities of t-PA and scu-PA, useful for in vitro, animal, and early clinical studies, were obtained initially from human cell lines grown in vitro: the Bowes melanoma cell line for t-PA and transformed human kidney cells for scu-PA. Both agents have subsequently been produced by recombinant DNA methods.

t-PA and scu-PA (scu-PA is available for clinical use only in Europe) display relative fibrin selectivity, albeit by entirely different mechanisms. The selectivity of t-PA derives from the presence of a fibrin binding site on the molecule. t-PA binds fibrin with a $K_d$ of 0.16 μM; when bound, its $K_{cat}$ for plasminogen activation decreases from 83 μM to 0.18 μM, and its $K_{cat}$ increases from 0.07 to 0.28 second$^{-1}$, resulting in an increase in catalytic efficiency of approximately 1,000-fold. Although scu-PA does not appear to bind directly to fibrin, it activates fibrin-bound plasminogen much more readily than the plasminogen present in plasma and results in a fibrin selectivity similar to that of t-PA.

When t-PA and scu-PA were discovered to possess intrinsic fibrin selectivity, it was reasoned that if the action of plasmin were largely confined to the thrombus, circulating plasma proteins would be spared, a lytic state would be avoided, and the probability of hemorrhage would diminish. As will become apparent later in this review, the validity of this hypothesis is not universally accepted.

Clinical studies have been conducted with single- and two-chain t-PA. Both forms are present in the circulation, and both can be produced in large quantities. Cleavage by plasmin of single-chain t-PA between amino acids Arg$^{175}$ and Ile$^{176}$ produces two-chain t-PA. There is no change in molecular size, and two-chain t-PA is similar in fibrin selectivity and catalytic activity (in vivo) to its single-chain precursor.

Urokinase is also synthesized and secreted by endothelial cells as a single polypeptide chain of 54,000 Da (scu-PA). Unlike single-chain t-PA, scu-PA does not possess plasminogen activator activity. Cleavage by plasmin between amino acids Lys$^{158}$ and Ile$^{159}$ activates the molecule but does not reduce its molecular size because the two constituent chains continue to be associated by a disulfide bond. Urokinase was first studied clinically in the low molecular weight, 33,000-Da form (a proteolytic fragment of two-chain 55,000-Da urokinase). Unlike scu-PA, neither high nor low molecular weight two-chain urokinase possesses fibrin selectivity or resistance to plasminogen activator inhibitors; consequently, both forms of two-chain urokinase must be considered first-generation plasminogen activators.

Streptokinase has now been chemically modified to form a third-generation plasminogen activator that has an increased fibrin specificity and a prolonged half-life in plasma. The plasminogen-streptokinase complex was formed in vitro, and then an essential serine in the catalytic site of plasminogen was acylated. APSAC does not possess enzymatic activity, but its plasminogen component is capable of binding to fibrin. Once bound to fibrin, deacylation occurs. Deacylation then enables the complex to activate other plasminogen molecules. APSAC was designed with two potential advantages in mind. First, it was hoped that the enzymatically inactive, acylated molecule would rapidly bind to fibrin, thereby bypassing the activation of circulating plasminogen. Once APSAC was associated with fibrin, deacylation would then permit the activation of fibrin-bound plasminogen, concentrating fibrinolysis at the clot and thereby spar-
ing circulating plasma proteins. In animal models in which fibrinogen sparing was used as a measure of fibrin selectivity, APSAC was significantly more fibrin-specific than urokinase or streptokinase but less so than t-PA.\textsuperscript{12} APSAC's second putative advantage over any of the available plasminogen activators is its longer half-life, which permits the administration of a thrombolytic dose in a single injection.

**Pharmacologic Effects of Plasminogen Activators**

Each plasminogen activator has a unique pharmacologic profile. Their physical half-lives in the human circulation cover a wide range. The half-lives of t-PA and scu-PA are very short (6 and 7 minutes, respectively\textsuperscript{11,9}), those of urokinase and streptokinase intermediate (16 and 23 minutes, respectively\textsuperscript{13,12}), and that of APSAC rather long (90 minutes\textsuperscript{12}). Functional half-life is equally as important as physical half-life. t-PA and two-chain urokinase are readily and irreversibly inhibited in the circulation by plasminogen activator inhibitor I. scu-PA, before conversion to two-chain urokinase, is resistant to the plasma inhibitors. Plasminogen activator inhibitor I, $\alpha_2$-antiplasmin, and $\alpha_2$-macroglobulin. Streptokinase, a bacterial protein, has no plasma inhibitors. Because APSAC contains modified streptokinase, it also cannot be inhibited directly.

The plasmin ultimately generated by the action of any plasminogen activator is susceptible to inhibition by $\alpha_2$-antiplasmin. The liver contains specific clearance receptors for t-PA, and scu-PA and uroki-

nase have hepatic and renal clearance. With an understanding of the specific determinants of clearance, it may be possible to use genetic engineering to prolong a plasminogen activator's functional or physical half-life. For example, receptor-mediated clearance of t-PA depends on amino acid sequence and glycosylation. Specific recognition sequences, as well as glycosylation sites, may be modified by site-directed mutagenesis.\textsuperscript{14}

**Plasminogen Activator Therapy for Acute Myocardial Infarction**

Unfortunately, because of the enormous intrinsic variation among patient populations and study designs, a reliable comparison among plasminogen activators concerning efficacy or side effects cannot yet be made. Here is what is presently known.

**Lysis of Coronary Thrombi**

Success in recanalization depends on the route of administration (intravenous or intracoronary), the age of the thrombus, and the dose of plasminogen activator. When administered directly into the coronary artery, streptokinase and t-PA are roughly equipotent in dissolving coronary thrombi. Intracoronary administration has now been abandoned, not only because of the personnel and number of catheterization suites this route of administration requires, but also because the duration of ischemia before recanalization is perhaps the most important prognostic issue in thrombolytic treatment. Mobilizing a catheterization team simply cannot be done as quickly as placing an intravenous line. Recent studies have focused on the intravenous administration of thrombolytic agents, but there are very few comparative data on the rates of recanalization. A single large study comparing recanalization rates by coronary arteriography indicates that t-PA is more effective than streptokinase in lysing thrombi.\textsuperscript{15} Some investigators argue that generalization from this study may not be appropriate, especially because lower doses of t-PA are now administered. The dilemma is that although lower doses reduce hemorrhagic complications (for t-PA as well as other plasminogen activators), lower doses also reduce the effectiveness of recanalization.

**Reduction of Mortality**

Far more significant than an objective demonstration of recanalization is the significant reduction in in-hospital and 1-year mortality when a plasminogen activator is administered soon after the onset of symptoms. There is also a significant preservation of left ventricular function. Administration of streptokinase within 4 hours of initial symptoms reduces mortality after myocardial infarction by 23–81\%,\textsuperscript{10–19} Similar, but not statistically significant, results have been obtained with urokinase in smaller-scale clinical trials.\textsuperscript{20,21} t-PA reduces mortality by 23–36\%.\textsuperscript{15,22,23} For all plasminogen activators, a clear inverse relation exists between the degree of benefit and the time between the onset of symptoms and the onset of therapy.\textsuperscript{15,17}

**Coronary Artery Reocclusion After Plasminogen Activator Therapy**

The rate of coronary artery reocclusion after successful recanalization may vary with the plasminogen activator used. Streptokinase has the lowest incidence of reocclusion,\textsuperscript{15,24} but its systemic use is always associated with a marked depletion of plasma clotting components and a reduction in blood viscosity (probably because of fibrinogen depletion).\textsuperscript{25} t-PA, which at lower doses results in less depletion of clotting components, is associated with a higher frequency of reocclusion.\textsuperscript{23,24} But when t-PA is given at higher doses or in the form of a low-dose, long-term infusion (both are situations that cause an increase in hemostatic defects), the reocclusion rate is dramatically reduced.\textsuperscript{24} Preliminary evidence suggests that reocclusion is not so frequent with scu-PA as with t-PA, but a direct comparison has yet to be reported. Although it has certainly not been proven, the available data suggest that when a plasminogen activator is used alone, the more severe the hemostatic defect, the less likely rethrombosis. Platelet-active agents given with plasminogen activators can also reduce the likelihood of rethrombosis. The results of the ISIS-II trial suggest that the addition of aspirin to streptokinase therapy affords benefit without increasing the risk of hemorrhage.\textsuperscript{26}
Hemorrhage in Patients Receiving Plasminogen Activator Therapy

Hemorrhage is the most feared complication of plasminogen activator therapy. Bleeding that is severe enough to require treatment primarily takes three forms: 1) intracranial bleeding, which occurs more commonly in patients with significant hypertension and which almost always results in major morbidity or in mortality; 2) gastrointestinal bleeding, which may occur more commonly in patients with occult gastritis or peptic ulcer disease and rarely causes mortality (although it frequently necessitates transfusion); and 3) bleeding from a site of arterial damage (most commonly from catheterization or arterial puncture), which can also necessitate transfusion but is rarely the cause of significant morbidity.

The precise relation between the lysis of a coronary thrombus and the event that results in hemorrhage at these sites is not clear. A thrombus that plugs a site of vascular injury from a needle or a peptic ulcer would most closely resemble a coronary thrombus; lysis of a thrombus at a site of vascular injury probably parallels that of a coronary thrombus. Although "fibrin plugs" have been advocated as the guardians of the vascular system's integrity at other sites, such as the central nervous system,2 direct evidence of their participation does not exist. Resolving this question is of considerable importance because interest in further developing thrombus-selective plasminogen activators hinges on the answer. If bleeding after plasminogen activator therapy is solely the result of fibrinolysis, then the relative fibrin selectivity of a plasminogen activator is irrelevant. If, on the other hand, hemorrhage occurs as a result of the severe disturbance in hemostasis known as the lytic state, fibrin-selective plasminogen activators would be of value. These activators would promote fibrinolysis at the site of the thrombus while avoiding the loss of clotting proteins.

Interestingly, no correlation seems to exist between the degree of fibrinogen or α2-antiplasmin depletion and the occurrence of cerebral hemorrhage.27 Because other important components of the clotting system are also lost, including platelet surface receptors,28–31 there is a need for clinical studies correlating a complete hemostatic profile with the incidence of hemorrhage.

Streptokinase, t-PA, and APSAC: Can They Be Compared?

Critical comparisons of t-PA and streptokinase are underway and will be reported within the next year. For the present, some general comparisons can be made. The reduction of mortality in myocardial infarction is similar for all agents.15,17,22,23,26,32 Recanalization is probably more efficient with t-PA15,22 or, possibly, APSAC22 than with streptokinase, and restenosis is probably more likely to occur with t-PA than with streptokinase.15,17,26 At comparable thrombolytic doses, there is less degradation of fibrinogen with t-PA than with streptokinase.15 And, as noted above, there are marked differences in half-life among the available plasminogen activators. Whether or not these differences have any importance beyond convenience remains to be determined.

Engineering New Plasminogen Activators Based on an Understanding of Protein Structure and Function

Native t-PA and scu-PA are single polypeptide chains. Normally, plasmin cleaves each at a unique site. In both proteins, the segment that is carboxyl terminal to the cleavage (the B chain) is responsible for catalytic activity. It has considerable amino acid sequence homology with other serine proteases such as trypsin and chymotrypsin; however, in contrast to these enzymes, t-PA and scu-PA are highly selective for plasminogen. The amino terminal segment of t-PA (the A chain) contains a fibrin binding site. Less is known about the A chain of scu-PA.

More recently, on the basis of the complete complementary (c)DNA and genomic DNA nucleotide sequences of t-PA and scu-PA (and their amino acid sequences, Figure 1), both molecules have been inferred to be multidomain proteins. The genomic DNA coding for t-PA is organized into multiple exons (coding regions) and introns (intervening regions). Each exon generally codes for a single protein domain. Knowledge of the genomic organization has facilitated the expression of t-PA variants lacking specific domains or groups of domains. Site-directed mutagenesis allowed even more discrete changes to be made in the molecule. An examination of the functional properties of such mutant proteins then permitted the assignment of specific roles for most of the domains. This examination also defined amino acid sequences and glycosylation sites essential for hepatic clearance.

t-PA has five discrete domains,33–35 Four are contained in the A chain, and the B chain comprises a single domain. The structures of the domains resemble, by sequence homology, those of other proteins. The A chain has a "finger" domain similar to that of fibronectin,36 an epidermal growth factor (EGF)-like domain homologous to those of urokinase, protein C, and coagulation factors IX and X,37 and two "kringle" domains joined by intradomain disulfide bonds similar to those found in plasminogen, scu-PA, and prothrombin. The fifth, B-chain domain is homologous to the catalytic regions of other serine proteases.

Detailed information about the function of the five domains has been obtained from studies of the properties of single-chain t-PA deletion mutants.33–35,38 The high-affinity interaction between t-PA and fibrin is mediated by the finger or EGF-like domain (or both). The stimulation of t-PA's catalytic activity by fibrin appears to depend on the presence of either or both kringles; the most recent data suggest that the two kringles act similarly.33 The inhibition of this activity by plasminogen activator inhibitor I
appears to depend on the inhibitor’s direct binding to the activator’s catalytic domain. The consensus, from a number of laboratories, is that the domains have autonomous but related functions.

Knowledge of the functional organization of scu-PA is less complete. The A chain of scu-PA contains, in addition to an EGF-like domain, a single kringle region that is considerably homologous with the kringles of t-PA (even though scu-PA does not appear to bind fibrin). The B chain of scu-PA contains the catalytic portion of the molecule. As indicated above, an important property that differentiates scu-PA from t-PA is scu-PA’s resistance to irreversible inhibition by plasminogen activator inhibitor I (and to other plasminogen activator inhibitors). Therefore, unlike t-PA, scu-PA is stable in human plasma for extended periods. (Plasminogen activator inhibitor I binds reversibly to scu-PA: when scu-PA forms a ternary complex with fibrin and plasminogen, plasminogen activator inhibitor I is displaced.) Whether this resistance to inhibition resides in the A or B chain of scu-PA is not clear; however, it has been demonstrated that the catalytic site becomes susceptible to irreversible inhibition after plasmin cleaves scu-PA between residues Lys$^{158}$ and Ile$^{159}$.

Modification of Native Plasminogen Activators by Recombinant DNA Methods

Because scu-PA is quite resistant to inhibition by plasminogen activator inhibitor I (a feature unique to scu-PA), considerable effort has been dedicated to elucidating and manipulating the mechanism of this resistance to inhibition. To this end, Stump et al.\textsuperscript{41} initially described a low molecular weight form of scu-PA that has subsequently been produced by recombinant DNA methods.\textsuperscript{42} This low molecular weight scu-PA contains amino acids 144 to 411 of the full-length molecule. It retains, however, the full-length molecule’s fibrin selectivity and resistance to plasminogen activator inhibitor I. In another approach, Nelles et al.\textsuperscript{43} attempted to prevent the conversion of single-chain urokinase to two-chain urokinase by mutating the Lys$^{158}$-Ile$^{159}$ peptide bond. Either Gly or Glu was substituted for Lys$^{158}$ by site-directed mutagenesis. Although the two mutant proteins could not be converted to two-chain urokinase, their $K_m$ for activation of plasminogen was much lower than that of native scu-PA, suggesting that the conversion to two-chain urokinase is important for optimal function in fibrinolysis.

Approaches to Increasing Fibrin Selectivity

Addition of Fibrin-Binding Domains to Native Plasminogen Activators

Once it became clear that plasminogen activators could be modified at specific sites in their structures, several laboratories began designing hybrid plasminogen activators that combined the desirable properties of two or more molecules. Robbins and coworkers\textsuperscript{44,45} attempted to add increased fibrin-binding ability to plasminogen activators by preparing hybrids combining the fibrin-binding domain of plasminogen and either the catalytic domain of urokinase or the catalytic domain of t-PA. The two parent molecules were linked chemically by formation of a disulfide bond. Robbins and coworkers demonstrated that fibrin-binding domains could be appended to either urokinase or t-PA; however, the
fibrinolytic potency of the hybrids was no greater than that of native scu-PA or t-PA.

Nelles et al\textsuperscript{42} used the tools of molecular biology to approach the problem of increasing fibrin selectivity. They constructed a recombinant hybrid protein containing the A chain (fibrin-binding domain) of t-PA and the low molecular weight form of scu-PA. They spliced the cDNA for t-PA, which encodes the 5' untranslated region and amino acids Ser\textsuperscript{1} through Thr\textsuperscript{209}, to the cDNA for low molecular weight scu-PA, which codes for Leu\textsuperscript{44} through Leu\textsuperscript{111}, and then expressed the hybrid DNA in Chinese hamster ovary cells. The fibrin-binding activity of this recombinant hybrid plasminogen activator was significantly less than that of native t-PA, as was its fibrinolytic potency.

**Antibody-Targeted Fibrinolysis**

The results of Stump et al\textsuperscript{41} Nelles et al\textsuperscript{42,43} and Robbins et al\textsuperscript{44,45} suggest that it is not possible to significantly increase the fibrin selectivity of native plasminogen activators by shuffling the domains of existing activators. Our laboratory has taken an alternative approach that focuses on increasing the selectivity of a plasminogen activator catalytic site by combining it with the unique selectivity of a fibrin-specific monoclonal antibody. Monoclonal antibodies were first developed that bind to fibrin with high affinity but not to fibrinogen.\textsuperscript{46} Then, these were linked by chemical, immunologic, or recombinant DNA methods to urokinase, t-PA, and, most recently, scu-PA.\textsuperscript{47-51} The linking methods are complementary. As a screening tool with which to determine the feasibility of the approach, chemical linkage was used to combine the plasminogen activators with fibrin-specific antibody 59D8.\textsuperscript{47-50} With results indicating increased potency and selectivity, it seemed reasonable to engineer by recombinant DNA techniques a single molecule that possessed a fibrin-specific antibody binding site and a plasminogen activator catalytic site.

Our laboratory has taken another approach that uses bifunctional antibodies (with dual specificity for fibrin and a plasminogen activator) to increase the concentration of plasminogen activator in the proximity of fibrin. It was hoped that this approach could be used to increase the potency of intrinsic plasminogen activators already present in the circulation.

**Hybrid Molecules Containing an Antifibrin Antibody and a Plasminogen Activator**

Chemically conjugated model compounds have been assembled by linking antifibrin antibody 59D8 (or its Fab) to urokinase or t-PA by means of the disulfide cross-linking reagent N-succinimidyl 3-(2-pyridyldithio)propionate.\textsuperscript{47,48} The urokinase-59D8 and t-PA–59D8 conjugates were significantly more potent than either urokinase or t-PA in several in vitro assays\textsuperscript{49} and in vivo in the rabbit jugular vein model.\textsuperscript{49} The activity of the model compounds was also highly specific in vitro and in vivo, and the increase in fibrinolytic potency was accompanied by a decrease in the degradation of plasminogen, $\alpha_{2}$-antiplasmin, and fibrinogen.

With the success of these chemically conjugated model compounds, recombinant DNA methods were used to combine a fibrin-specific antibody binding site with a plasminogen activator catalytic site. We have now tested two such recombinant, activator-antibody constructs. The first contained the genomic DNA encoding the heavy chain of antibody 59D8 and a cDNA sequence encoding the light chain of t-PA. The recombinant molecule was expressed as a dimer containing two modified antibody molecules with two t-PA light chains. Although it bound fibrin with an affinity equal to that of antibody 59D8 and activated plasminogen in a manner similar to that of native t-PA, the chimeric molecule was ineffective in lysing a plasma clot. Subsequent evaluation of this molecule has demonstrated that a plasmin cleavage site absent in the recombinant protein, but present in native t-PA, may have prevented the fibrin-mediated enhancement of catalysis that occurs normally with native t-PA.

A second construct (now partially characterized) contains the heavy and light chains of antibody 59D8 and the low molecular weight form of scu-PA rather than the light chain of t-PA. This molecule does contain a plasmin-sensitive bond (in this case, the Lys\textsuperscript{59}-Ile\textsuperscript{59} bond in scu-PA) and, in contrast to recombinant t-PA–59D8, recombinant scu-PA–59D8 appears in preliminary studies to be an effective fibrinolytic agent.\textsuperscript{52}

**Bispecific Antibodies**

Bispecific antibodies are capable of binding two different antigens and thereby of bringing them close to one another.\textsuperscript{53} Model bispecific antibodies have been synthesized by chemically cross-linking antifibrin antibody 59D8 to an antibody specific for scu-PA (PEG)\textsuperscript{54} or an antibody specific for t-PA (TCL8).\textsuperscript{55} The conjugate in which 59D8 is linked to TCL8 ($K_a$, $5 \times 10^{-7}$ M) is best characterized.\textsuperscript{55} We have also synthesized bispecific antibodies by the hybrid-hybridoma method,\textsuperscript{56} which are uniform, stable on storage, and easy to produce. Both the chemically linked and hybrid-hybridoma antifibrin anti-t-PA antibodies can effectively concentrate t-PA to fibrin in vitro and produce significant fibrinolysis with very small amounts of t-PA. The chemically linked bispecific antibody has also been tested in the rabbit jugular vein model. It increased the effectiveness of t-PA in thrombolysis while sparing fibrinogen and $\alpha_{2}$-antiplasmin.\textsuperscript{55}

**Conclusion**

There is little doubt that plasminogen activators are an important adjunct to the treatment of acute myocardial infarction. In many patients, benefit outweighs risk. But there is the disquieting potential, as a result of treatment, for a fatal or debilitating cerebral hemorrhage to occur with a probability of between 0.5%
and 1%; mortality from the infarction itself is only about 10%. It must be established whether hemorrhage is an intrinsic risk of fibrinolytic therapy, regardless of the selectivity of the agent used, or whether increased selectivity for a component of the thrombus is of value in diminishing hemorrhagic complications. Fibrin-selective and nonselective plasminogen activators are now being rigorously compared in large multicenter trials, and it would seem prudent to await the results of the ISIS-III and GISSI-II studies. These studies address not only the relative merits of the agents being tested but also the desirability of designing and clinically testing newer agents, agents in which selectivity for a component of the thrombus is further increased.

Our bias is that selectivity will be recognized as a desirable property. The ideal plasminogen activator would be designed to recognize the coronary thrombus, to act locally in lysing it, and to then be eliminated or inactivated without generally perturbing the hemostatic system. Although some available agents possess various degrees of fibrin selectivity, all have at times caused a lytic state (with extensive loss of clotting proteins), and all have caused serious hemorrhage. Can the coronary thrombus be differentiated from thrombi in other sites? Is cerebral hemorrhage really related to thrombolysis? Or is some other process more indirectly related to the loss of an intact hemostatic system responsible? When these questions are answered, the tools of protein engineering can be used to create molecules that have optimal properties with regard to both recognition of components of the thrombus and duration of action. For example, it may be possible to differentiate between recently formed and older thrombi on the basis of relative degrees of fibrin cross-linking. One would hope that a coronary thrombus that is characterized by acute ischemic pain would be of more recent origin than a silent fibrin plug that holds back potential bleeding. Those components that have been modified by the clotting process offer the most easily defined targets toward which to direct a clot-selective agent. Fibrin, which differs in its covalent structure from its circulating precursor fibrinogen, is one obvious choice. Alternative targets include platelets altered during the clotting process, epitopes comprising the junction between platelet receptors and the proteins that bind to them (such as von Willebrand’s factor, fibrinogen, and fibronectin28–30), and the covalent link between α2-antiplasmin and fibrin. There is also a growing understanding of the determinants that govern the clearance of a protein from the circulation. By appropriate engineering, it may also be possible to impart a circulatory half-life that is consistent with the therapeutic goals of clot dissolution and prevention of recocclusion yet avoids the risk of an excessively long impairment of the hemostatic system.

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