Safety and Efficacy of the Various Thrombolytic Agents

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Thrombolytic agents are in widespread use for the dissolution of arterial and venous pathologic thrombi. Clinical settings where thrombolysis has played an important role include the acute coronary syndromes, peripheral arterial occlusion, ischemic stroke, deep venous thrombosis, and pulmonary embolism. Thrombolytic agents have been successfully employed in each of these areas, achieving dissolution of the occluding thrombus, reconstitution of blood flow, and improvement in the status of the tissue bed supplied or drained by the involved vascular segment. All clinically available thrombolytic agents act through cleavage of the plasminogen molecule to its active form, plasmin. Despite this similar mechanism of action, the thrombolytic agents differ in several biochemical parameters, including fibrin specificity, fibrin affinity, and relative resistance to inactivating factors in the plasma. Whether these differences account for significant differences in clinical outcome is a matter of some dispute. It is quite possible that in vitro biochemical differences do not have meaningful clinical correlates. However, there exists some evidence to suggest that differences in the risk of distant hemorrhage, idiosyncratic reactions, and the rapidity of clot dissolution do exist. An ideal agent for peripheral vascular thrombolysis would be one that was specific in its actions at the site of pathologic thrombi yet left the important and desirable pathologic thrombi that seal vascular defects unscathed. Although such an agent has not yet been identified, an understanding of the mechanism of action and principles underlying pharmacologic thrombolysis provides the necessary foundation of knowledge to choose a particular thrombolytic agent for a given clinical scenario.
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Although thrombolytic agents have been used clinically for over 50 years, and although most of the currently available agents have been used for over a decade, there exists a dearth of objective information comparing one agent to another. Such information is vital to clinicians when making choices about the most appropriate thrombolytic agent for a particular clinical situation. In the absence of objective comparative data, an understanding of the

Table 1
Characterization of Fibrinolytic Components

Component	Molecular Weight	Plasma $t_{1/2}$	Properties
Streptokinase	48,000	16 min/90 min	Complexes with plasmin(ogen) to gain activity
Urokinase	32,000/54,000	14 min	Direct plasminogen activator
r-Urokinase*	54,000	7 min	Similar in most respects to natural urokinase
r-Prourokinase*	49,000	7 min	Little intrinsic activity, converted to urokinase
rt-PA	68,000	3.5 min	Exhibits great degree of fibrin affinity and specificity
TNK-rt-PA	65,000	15 min	A modified rt-PA with a longer half-life, greater fibrin specificity and greater resistance to PAI-1
Reteplase	39,000	14 min	A truncated rt-PA with a longer half-life
Plasminogen	88,000	2.2 days	Binds to fibrin, converted to active plasmin
Plasmin	88,000	0.1 sec	Serine protease that cleaves fibrin
α-2-Antiplasmin	70,000	3 days	Inactivates free plasmin
PAI-1	52,000	Unknown	Inactivates the plasminogen activators

rt-PA, recombinant tissue plasminogen activator; PAI, plasminogen activator inhibitor.
*Not commercially available.

biochemical properties of the agents and a review of the anecdotal and retrospective data will allow one to make rational decisions for patients presenting with peripheral vascular occlusions.

Pharmacology of Thrombolytic Agents

All clinically available thrombolytic agents in clinical use are *plasminogen activators* (Table 1). As such, they do not directly degrade fibrinogen. Rather, they are trypsin-like serine proteases that have high specific activity directed at the cleavage of a single peptide bond in the plasminogen zymogen, converting it to plasmin. Plasmin is the active molecule that cleaves fibrin polymer to cause the dissolution of thrombus. Milstone first recognized the importance of plasminogen in 1941, when it was noted that clots formed with highly purified fibrinogen and thrombin were not lysed by streptococcal fibrinolysin unless a small amount of human serum (plasminogen) was added.[1] Recognizing this direct role of plasminogen, early investigators attempted to dissolve occluding thrombi with the administration of exogenous plasmin. Free plasmin, however, was ineffective as a thrombolytic agent, accounting for the failure of these attempts. Effective thrombolysis can be achieved only when *fibrin-bound* plasminogen is converted to its active form of plasmin at the site of the thrombus.[2]

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The dependence of fibrinolysis on adequate circulating levels of plasminogen is best illustrated by studies of the fibrinolytic potential of blood drawn from patients receiving intravenous administration of thrombolytic agents for acute myocardial infarction.[3] Blood obtained soon after the start of thrombolytic administration displayed a great degree of in vitro fibrinolytic potential. Aliquots of plasma drawn from the patients and then added to radiolabeled clots in test tubes produced rapid dissolution of the clots. By contrast, similar aliquots drawn from patients after 20 minutes of thrombolytic administration had considerably less thrombolytic potential. The explanation for this observation relates to the amount of plasminogen present in the blood. Prolonged thrombolytic administration consumed all

Table 2
Properties of Thrombolytic Agents

Agent	Fibrin Specificity	Fibrin Affinity
Streptokinase	Low	Low
APSAC*	Low	Intermediate
Urokinase	Low	Low
Pro-urokinase*	High	Low
rt-PA	High	High
TNK-rt-PA	Very High	High
Reteplase (r-PA)	Moderate	Low
Bat-PA*	Very High	Low

APSAC, anisoylated plasminogen streptokinase activator complex; rt-PA, recombinant tissue plasminogen activator; Bat-PA, bat plasminogen activator.
*Not commercially available.

of the endogenous plasminogen and, despite continued administration of thrombolytic agent, no further clot lysis was possible.

Classification of Thrombolytic Agents

Several schemes may be used to classify thrombolytic agents. The agents may be grouped by their mechanism of action—those that directly convert plasminogen to plasmin versus those that are inactive zymogens and require transformation to an active form before they can cleave plasminogen. Thrombolytic agents can be grouped by their mode of production—those that are manufactured via recombinant techniques and those that are of bacterial origin. Of interest is that recombinant agents harvested from a bacterial expression system such as *Escherichia coli* do not contain carbohydrates, whereas products of mammalian hybridoma (eg, recombinant prourokinase from mouse hybridoma SP2/0 cells) are fully glycosylated.

Thrombolytic agents can be classified by their pharmacologic actions—those that are "fibrin-specific" (bind to fibrin but not fibrinogen) versus those that are nonspecific and those that have a great degree of "fibrin affinity" (bind avidly to fibrin) versus those that do not (Table 2). We have found it most useful to classify thrombolytic agents into groups based on the origin of the parent compound. It is most efficient to divide the agents into four groups: the streptokinase compounds, the urokinase compounds, the tissue plasminogen activators, and an additional, miscellaneous group consisting of novel agents that are distinct from agents in the three other groups.

Streptokinase Compounds

Streptokinase (SK), originating from the *Streptococcus* bacteria, was the first thrombolytic agent to be described but is now used only on a limited basis.[4] SK is a 50,000 daltons molecule with a biphasic half-life comprising a rapid $t^{1/2}$ of 16 minutes and a second, slower $t^{1/2}$ of 90 minutes. Whereas the initial half-life is accounted for by complexing of the molecule with SK antibodies, the second half-life represents the actual biologic elimination of the protein.

SK differs from other thrombolytic agents with respect to the stoichiometry of plasminogen binding. Whereas other agents directly convert plasminogen to plasmin, SK must form an equimolar stoichiometric complex with a plasmin or plasminogen molecule to gain activity. Only then can this SK-plasmin(ogen) complex activate a second plasminogen molecule to form active plasmin; thus two plasminogen molecules are utilized in SK-mediated plasmin generation.

SK suffers from the limitation of antigenic potential. Preformed antibodies exist to a certain extent in all patients who have been infected with the *Streptococcus* bacterium. Similarly, patients with exposure to SK may have high antibody titers on repeat exposure. These neutralizing antibodies inactivate exogenously administered SK. SK antibodies may be overwhelmed through the use of a large initial bolus of drug, and a large initial SK loading dose may be employed in this regard. Some investigators have recommended measurement of antibody titers prior to beginning SK therapy, gauging the loading dose on the basis of this titer.[9]

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SK administration is complicated by allergic reactions in approximately 2% of patients treated, with

the development of urticaria, periorbital edema, and bronchospasm.[10] Pyrexia may also occur but is usually adequately treated with acetaminophen. The major untoward effect associated with SK is hemorrhage. SK-associated hemorrhage may be no different from bleeding associated with any thrombolytic agent. The primary cause is likely systemic actions of the agent on the thrombi sealing the sites of vascular disintegrity. The generation of free plasmin, however, can contribute to the problem, with degradation of fibrinogen and other serum clotting proteins as well as the release of fibrin(ogen)-degradation products that are potent anticoagulants themselves and exacerbate the coagulopathy.

Recognizing the potential limitations with SK, anisoylated plasminogen-streptokinase activator complex (APSAC) was developed by pharmacologists at Beecham Laboratories.[11] APSAC has a longer half-life than SK, because acylation renders the complex less susceptible to degradation. Because of this property, it was anticipated that APSAC would be associated with a reduced risk of rethrombosis. Contrary to expectations, APSAC offered little clinical benefit over SK or recombinant tissue plasminogen activator (rt-PA) when studied in the setting of acute coronary occlusion;[12] at present APSAC is not used to treat thrombi in the peripheral vasculature.

Urokinase Compounds

The fibrinolytic potential of human urine was first described by Macfarlane and Pilling in 1947.[13] The active molecule was extracted, isolated, and named "urokinase" (UK) in 1952.[14] This urokinase-type plasminogen activator is a naturally occuring serine protease composed of two polypeptide chains occurring in a low molecular weight (32,000 daltons) and high molecular weight (54,000 daltons) form. The high molecular weight form predominates in UK isolated from urine, whereas the low molecular weight form predominates in UK obtained from tissue culture of kidney cells. Unlike SK, UK directly activates plasminogen to form plasmin; prior binding to plasminogen or plasmin is not necessary for activity. Also in contrast to SK, preformed antibodies to UK are not observed. The agent is nonantigenic, and untoward reactions of fever or hypotension are rare.

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Presently, the only UK employed in the United States is of tissue-culture origin, manufactured from human neonatal kidney cells (Abbokinase, Abbott Laboratories).

Abbokinase is indicated for the lysis of pulmonary embolism. However, it has been used extensively for peripheral vascular occlusions. UK has been fully sequenced, and a recombinant form of UK (r-UK) was tested in a single trial of patients with acute myocardial infarction (MI) and in two multicenter trials of patients with peripheral arterial occlusion.[15] r-UK is fully glycosylated because it is derived from a murine hybridoma cell line. r-UK differs from Abbokinase in several respects. First, r-UK has a higher molecular weight than Abbokinase. Second, r-UK has a shorter half-life than its low-molecular-weight counterpart. Despite these differences, however, the clinical effects of the two agents have been quite similar.

A precursor of UK was discovered in urine in 1979.[16] Prourokinase was characterized and subsequently manufactured by recombinant technology using *Escherichia coli* (nonglycosylated) or mammalian cells (fully glycosylated). This single-chain form is an inactive zymogen inert in plasma but can be activated by kallikrein or plasmin to form active two-chain UK. This property accounts for amplification of the fibrinolytic process—as plasmin is generated, more prourokinase is converted to active UK, and the process is repeated. Prourokinase is relatively fibrin-specific, that is, its fibrin-degrading (*fibrinolytic*) activity greatly outweighs its fibrinogen-degrading (*fibrinogenolytic*) activity. This feature is explained by the preferential activation of fibrin-bound plasminogen found in a thrombus over free plasminogen found in flowing blood. Nonselective activators such as SK and UK activate free and bound plasminogen equally and induce systemic plasminemia, with resultant fibrinogenolysis and degradation of factors V and VII.

Given the potential advantages of prourokinase over UK, Abbott Laboratories produced a recombinant form of prourokinase (r-proUK) from a murine hybridoma cell line. This recombinant agent, Prolyse (Abbott Laboratories), is converted to active two-chain UK by plasmin and kallikrein. Prolyse has been studied in the settings of MI, stroke, and peripheral arterial occlusion.

Tissue Plasminogen Activators

Tissue plasminogen activators (rt-PA) are a naturally occurring fibrinolytic agent produced by endothelial cells that is intimately involved in the balance between intravascular thrombogenesis and thrombolysis. Natural

rt-PA is a single-chain (527 amino acid) serine protease with a molecular weight of approximately 65,000 daltons. Plasmin hydrolyses the Arg_{275}-Ile_{276} peptide bond, converting the single-chain molecule into a two-chain moiety. In contrast to most serine proteases (eg, UK), the single-chain form of rt-PA has significant activity.

rt-PA has theoretical benefits over other thrombolytic agents. The agent exhibits significant *fibrin specificity*. In plasma, the agent is associated with little plasminogen activation. At the site of the thrombus, however, the binding of rt-PA and plasminogen to the fibrin surface induces a conformational change in both molecules, greatly facilitating the conversion of plasminogen to plasmin and dissolution of the clot. rt-PA also manifests the property of *fibrin affinity*, that is, it binds strongly to fibrin. Other fibrinolytic agents such as prourokinase do not share this property of fibrin affinity. Fibrin specificity and fibrin affinity may, however, be theoretical benefits that play a more important role in systemic administration for acute myocardial infarction than they do for catheter-directed peripheral vascular thombolysis.

rt-PA was produced in the 1980s after molecular cloning techniques were used to express human rt-PA DNA. Activase (Genentech), a predominantly single-chain form of rt-PA, was eventually approved in the United States for the indications of acute MI and massive pulmonary embolism. rt-PA has been studied extensively in the setting of coronary occlusion. In the Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO)-I trial of approximately 41,000 patients with acute MI, rt-PA was more effective than SK in achieving vascular patency.[18] Despite a slightly greater risk of intracranial hemorrhage with rt-PA, overall mortality was significantly reduced.

In an effort to lengthen the duration of bioavailability of rt-PA, the molecule was systematically bioengineered. Initial investigations identified regions in kringle 1 and the protease portion of rt-PA that mediated hepatic clearance, fibrin specificity, and resistance to plasminogen activator inhibitor. Three sites were modified to create TNK-rt-PA, a novel molecule with a greater half-life and fibrin specificity. The longer half-life of TNK-rt-PA allows successful administration as a single bolus, in contrast to the requirement for an infusion with rt-PA. In addition, TNK-rt-PA manifests greater fibrin specificity than rt-PA, resulting in less fibrinogen depletion. In studies of acute coronary occlusion, TNK-rt-PA performed at least as well as rt-PA, concurrent with greater ease of administration.[19]

Similar to TNK-rt-PA, the novel recombinant plasminogen activator reteplase comprises the kringle 2 and protease domains of rt-PA. Reteplase (r-PA) (Retevase, Centocor) was developed with the goal of avoiding the necessity of a continuous intravenous infusion, thereby simplifying ease of administration. r-PA, produced in *Escherichia coli* cells, is nonglycosylated, demonstrating a lower fibrin-binding activity and a diminished affinity to hepatocytes. This latter property accounts for a longer half-life than rt-PA, potentially enabling bolus injection versus prolonged infusion for acute MI. The fibrin affinity of r-PA is only 30% of that exhibited with rt-PA. The decrease in fibrin affinity is hypothesized to reduce the incidence of distant bleeding complications in MI, in a manner similar to that of SK over rt-PA in the GUSTO trial.[20] In fact, several properties of r-PA may account for a decreased risk of hemorrhage, including poor lysis of platelet-rich, older clots.[21] Again, theoretical advantages may play a more important role in the setting of acute MI than they do in peripheral arterial occlusion requiring long continuous infusions. To date, r-PA has been studied in the coronary setting alone, and has demonstrated some benefit over rt-PA in the Reteplase and Alteplase Patency Investigation During Myocardial Infarction (RAPID) 1[22] and RAPID 2[23] studies, as well as in GUSTO III.[24]

It appears that r-proUK offers the advantages associated with an agent that does not originate from a human cell source.

Prior to the late 1990s, there was scant data to provide a safe and efficacious dose for either rt-PA or r-PA. After UK became unavailable, clinicians gained experience with these two alternative agents. As time went on, clinicians began to investigate lower and lower doses of these two agents in an attempt to reduce major bleeding complications. At this point in time, clinicians appear to feel most comfortable with doses of rt-PA that vary between 0.5 and 2.0 mg/hour. Clinicians have also settled on a dose of r-PA that varies between 0.25 and 1.0 U/hour. However, there currently remains a lack of consensus regarding the optimal dosing regimen for these agents.

Miscellaneous Agents

A wide variety of novel thrombolytic agents exist, all of which have undergone extensive preclinical study, but few of which have been

adequately evaluated in patients. Vampire bat plasminogen activator ("bat PA"), was cloned and expressed from the saliva of the vampire bat *Desmodus rotundus*.[25] This agent manifests extraordinary fibrin specificity; the plasminogenolytic activity is over 100,000 times greater in the presence of fibrin. The half-life of bat PA is 5 to 9 times slower than that of rt-PA, offering some potential advantages with respect to ease of administration. To date, clinical trials have been limited to Phase I study with healthy volunteers.[26]

Fibrolase is a metalloproteinase originating from venom of the southern copperhead snake.[27] Fibrolase is a unique fibrinolytic agent that does not require plasminogen for its activity. Rather, the agent directly degrades fibrin without the requirement of any other blood components.

Staphylokinase is a by-product of the *Staphylococcus aureus* bacterium, originally mentioned in the classic streptococcal fibrinolysin paper of Tillett and Garner in 1933.[4] Staphylokinase has been produced by recombinant techniques and has been studied in the settings of MI, peripheral arterial occlusion, and deep venous thrombosis.[28] Like SK, staphylokinase is inactive and must bind to plasminogen to activate other plasminogen molecules. Unlike SK, staphylokinase is relatively fibrin-specific and spares circulating plasminogen and fibrinogen. Unfortunately, staphylokinase is antigenic, although less so with certain recombinant mutants.

Comparison of the Agents in Studies of Peripheral Vascular Disease

To date, there have been few well-designed clinical comparisons of the various thrombolytic agents in the peripheral vasculature. A variety of in vitro studies and retrospective clinical trials have been performed, and most point to improved efficacy and safety of UK and rt-PA over SK.[29,30] In an analysis of data collected in a prospective, single-institution registry at the Cleveland Clinic Foundation, UK demonstrated a diminished rate of bleeding complications when compared with rt-PA.[31] Efficacy was not evaluated in this trial.

There have been two prospective, randomized comparisons of UK and rt-PA. Neither was blinded. Meyerovitz and associates from the Brigham and Women's Hospital randomized 32 patients with peripheral arterial or bypass graft occlusions of less than 90 days duration to rt-PA (10 mg bolus, 5 mg/hr to a maximum of 24 hr) or UK (60,000 IU bolus, 4,000 IU/min for 2 hr, 2000 IU/min for 2 hr, then 1000 IU/min to a maximum of 24 hr total administration).[32] There was significantly greater systemic fibrinogen degradation in the rt-PA group (P = .01), indicating that the fibrin specificity of rt-PA was lost at this dosing regimen. rt-PA patients achieved more rapid initial thrombolysis, but efficacy was identical in the two groups by 24 hours. The trade-off to more rapid thrombolysis was a trend toward a higher rate of bleeding complications in the rt-PA–treated patients (P = .39).

The second randomized comparison of UK and rt-PA was the Surgery of Thrombolysis for the Ischemic Lower Extremity (STILE) trial, a three-armed multicenter comparison of UK (250,000 IU bolus, 4000 IU/min for 4 hr, then 2000 IU/min

Main Points

- Effective thrombolysis can be achieved only when fibrin-bound plasminogen is converted to its active form of plasmin at the site of the thrombus.
- Urokinase directly activates plasminogen to form plasmin, so prior binding to plasminogen or plasmin is not necessary for activity; preformed antibodies are not observed, and the agent is nonantigenic and untoward reactions of fever or hypotension are rare.
- Streptokinase, originating from the *Streptococcus* bacteria, was the first thrombolytic agent to be described but is now used only on a limited basis.
- Tissue plasminogen activator is a naturally occurring fibrinolytic agent produced by endothelial cells and intimately involved in the balance between intravascular thrombogenesis and thrombolysis.
- Tissue plasminogen activator exhibits significant fibrin specificity, greatly facilitating the conversion of plasminogen to plasmin and dissolution of the clot, and fibrin affinity, that is, it binds strongly to fibrin, unlike other fibrinolytic agents.
- The prevailing notion that recombinant tissue plasminogen activator is a quicker thrombolytic agent than urokinase is poorly documented in the literature. In fact, urokinase may provide an acceptable balance between thrombolytic efficacy and safety.

for up to 36 hr), rt-PA (0.05 to 0.1 mg/kg/hr for up to 12 hr) and primary operation.[33] There was 1 intracranial hemorrhage in the UK group (0.9%) and 2 in the rt-PA group (1.5%; no significant difference). Although actual rates of overall bleeding complications and efficacy were not reported for the two thrombolytic groups, the authors remarked that there were no significant differences detected in any of the outcome variables. In a subsequent "reanalysis" of the data, reported in 1999, the frequency of complete clot lysis was similar with urokinase and rt-PA at the time of the early arteriographic study.[34] This recent data suggests that the rate of thrombolysis may be quite similar, in direct contradistinction to the popularly held view that rt-PA is a much more rapidly acting agent. If, indeed, rt-PA were no more *efficacious* than UK, an increased risk of bleeding as demonstrated by Swischuk and associates[35] and Arepally and associates,[36] would not be balanced by faster thrombus degradation.

A multicenter, blinded trial compared the results of thrombolysis with UK versus r-UK in 300 patients with peripheral arterial occlusion. This data was never published (Abbott Laboratories, unpublished data). There were no significant differences noted between the two agents. A North American multicenter trial[37] compared three different doses of r-proUK to UK in 213 patients with lower-extremity arterial occlusions of less than 14 days duration. Whereas the higher r-proUK dose was associated with slightly greater percentage of patients with complete (> 95%) clot lysis at 8 hours, there was a mild increase in the rate of bleeding complications compared with either the UK or the lower-dose r-proUK groups. The fibrinogen levels fell in the higher r-proUK group, suggesting that fibrin specificity is lost at the higher-dose regimens for this compound.

Summary

In summary, the prevailing notion that rt-PA is a quicker thrombolytic agent than UK is poorly documented in the literature. In fact, UK may provide an acceptable balance between thrombolytic efficacy and safety. This balance likely explains the previous preference for UK as the agent of choice for peripheral vascular occlusions in the United States. New agents, such as r-proUK, may provide advantages over present agents, with improved efficacy and possibly increased safety when used for peripheral vascular thrombosis. ■

References

1. Milstone H. A factor in normal human blood which participates in streptococcal fibrinolysis. *J Immunol*. 1941;42:116.
2. Alkjaersig N, Fletcher AP, Sherry S. The mechanism of clot dissolution by plasmin. *J Clin Invest*. 1959;38:1086.
3. Onundarson PT, Haraldsson HM, Bergmann L, et al. Plasminogen depletion during streptokinase treatment or two-chain urokinase incubation correlates with decreased clot lysability ex vivo and in vitro. *Thromb Haemost*. 1993;70:998–1004.
4. Tillett WS, Garner RL. The fibrinolytic activity of hemolytic streptococci. *J Exp Med*. 1933;58:485.
5. Tillett WS, Sherry S. The effect in patients of streptococcal fibrinolysin (streptokinase) and streptococcal desoxyribonuclease on fibrinous, purulent, and sanguinous pleural exudations. *J Clin Invest*. 1949;28:173.
6. Tillett WS, Johnson AJ, McCarty WR. The intravenous infusion of the streptococcal fibrinolytic principle (streptokinase) into patients. *J Clin Invest*. 1955;34:169–185.
7. Cliffton EE, Grunnet M. Investigations of intravenous plasmin (fibrinolysin) in humans. *Circulation*. 1956;14:919.
8. Cliffton EE. The use of plasmin in humans. *Ann N Y Acad Sci*. 1957;68:209–229.
9. Jostring H, Barth U, Naidu R. Changes of antistreptokinase titer following long-term streptokinase therapy. In: Martin M, Schoop W, Hirsh J, eds. *New Concepts of Streptokinase Dosimetry*. Vienna: Hans Huber; 1978:110.
10. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomized trial of intravenous streptokinase, oral aspirin, both or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. *Lancet*. 1988;332:349.
11. Smith RAG, Dupe RJ, English PD, Green J. Fibrinolysis with acyl-enzymes: a new approach to thrombolytic therapy. *Nature*. 1981;290:505.
12. ISIS-3 Collaborative Group. ISIS-3: a randomized comparison of streptokinase vs tissue plasminogen activator vs anistreplase and of aspirin plus heparin vs aspirin alone among 41,299 cases of suspected acute myocardial infarction. *Lancet*. 1992;339:753.
13. Macfarlane RG, Pinot JJ. Fibrinolytic activity of normal urine. *Nature*. 1947;159:779.
14. Sobel GW, Mohler SR, Jones NW, et al. Urokinase: an activator of plasma fibrinolysin extracted from urine. *Am J Physiol*. 1952;171:768–769.
15. Credo RB, Burke SE, Barker WM, et al. Recombinant urokinase (r-UK): biochemistry, pharmacology, and clinical experience. In: Sasahara AA, Loscalzo J, eds. *New Therapeutic Agents in Thrombosis and Thrombolysis*. New York: Marcel Dekker, Inc.; 1997:513–537.
16. Husain SS, Lipinski B, Gurewich V, inventors; Isolation of plasminogen activators useful as therapeutic and diagnostic agents (single-chair, high-fibrin affinity urokinase).4,381,346. 1979. Patent application.
17. Ouriel K, Kandarpa K, Schuerr DM, et al. Prourokinase vs. urokinase for recanalization of peripheral occlusions, safety and efficacy: the PURPOSE trial. *J Vasc Interv Radiol*. 1999;10:1083–1091.
18. The GUSTO Investigators. An international randomized trial comparing four thrombolytic therapies for acute myocardial infarction. *N Engl J Med*. 1993;329:673–682.
19. Cannon CP, Gibson CM, McCabe CH, et al. TNK-tissue plasminogen activator compared with front-loaded alteplase in acute myocardial infarction: results of the TIMI 10B trial. Thrombolysis in Myocardial Infarction (TIMI) 10B Investigators. *Circulation*. 1998;98:2805–2814.
20. The GUSTO Investigators. An angiographic study within the global randomized trial of aggressive versus standard thrombolytic strategies in patients with acute myocardial infarction. *N Engl J Med*. 1993;329:1615.
21. Meierhenrich R, Carlsson J, Seifried E, et al. Effect of reteplase on hemostasis variables: analysis of fibrin specificity, relation to bleeding complications and coronary patency. *Int J Cardiol*. 1998;65:57–63.
22. Reteplase angiographic phase II international dose finding studies (RAPID I). *Circulation*. 1995;91:2725–2732.
23. Reteplase and alteplase patency investigation during myocardial infarction (RAPID II). *Circulation*. 1996;94:891–898.
24. Anonymous. A comparison of reteplase with alteplase for acute myocardial infarction. The global use of strategies to open occluded coronary arteries (GUSTO III) investigators. *N Engl J Med*. 1997;337:1118–1123.
25. Gardell SL, Duong LT, Diehl RE. Isolation, characterization and cDNA cloning of a vampire bat salivary plasminogen activator. *J Biol Chem*. 1989;264:17947–17952.
26. Schleuning WD, Bhargava A, Donner P.

Desmodus rotundus (vampire bat) plasminogen activator DSPAa1: a superior thrombolytic created by evolution. In: Sasahara AA, Loscalzo J, eds. *New Therapeutic Agents in Thrombosis and Thrombolysis*. New York: Marcel Dekker, Inc.; 1997:603–623.
27. Ahmed NK, Tennant KD, Markland FS, Lacz JP. Biochemical characteristics of fibrolase, a fibrinolytic protease from snake venom. *Haemostasis*. 1990;20:147–154.
28. Heymans S, Vanderschueren S, Verhaeghe R, et al. Outcome and one year follow-up of intra-arterial staphylokinase in 191 patients with peripheral arterial occlusion. *Thromb Haemost*. 2000;83:666–671.
29. van Breda A, Robison JC, Feldman L, et al. Local thrombolysis in the treatment of arterial graft occlusions. *J Vasc Surg*. 1984;1:103–112.
30. Ouriel K, Welch EL, Shortell CK, et al. Comparison of streptokinase, urokinase, and recombinant tissue plasminogen activator in an in vitro model of venous thrombolysis. *J Vasc Surg*. 1995;22:593–597.
31. Ouriel K, Gray BH, Clair DG, Olin JW. Complications associated with the use of urokinase and recombinant tissue plasminogen activator for catheter-directed peripheral arterial and venous thrombolysis. *J Vasc Interv Radiol*. 2000;11:295–298.
32. Meyerovitz M, Goldhaber SZ, Reagan K, et al. Recombinant tissue-type plasminogen activator versus urokinase in peripheral arterial and graft occlusions: a randomized trial. *Radiology*. 1990;175:75–78.
33. Anonymous. Results of a prospective randomized trial evaluating surgery versus thrombolysis for ischemia of the lower extremity. The STILE trial. *Ann Surg*. 1994;220:251–266.
34. Comerota AJ. A reanalysis of the STILE data. Personal communication.
35. Swischuk JL, Fox PF, Young K, et al. Transcatheter intraarterial infusion of rt-PA for acute lower limb ischemia: results and complications. *J Vasc Interv Radiol*. 2001;12(4):423-430.
36. Arepally A, Hofmann LV, Kim HS, et al. Weight-based rt-PA thrombolysis protocol for acute native arterial and bypass graft occlusions. *J Vasc Interv Radiol*. 2002;13(1):45-50.
37. Ouriel K, Veith FJ, Sasahara AA. Thrombolysis or peripheral arterial surgery: phase I results. *J Vasc Surg*. 1996;23:64–73.