

THE PRESENT AND FUTURE

REVIEW TOPIC OF THE WEEK

Therapeutic Fibrinolysis

How Efficacy and Safety Can Be Improved



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ABSTRACT

Therapeutic fibrinolysis has been dominated by the experience with tissue-type plasminogen activator (t-PA), which proved little better than streptokinase in acute myocardial infarction. In contrast, endogenous fibrinolysis, using one-thousandth of the t-PA concentration, is regularly lysing fibrin and induced Thrombolysis In Myocardial Infarction flow grade 3 patency in 15% of patients with acute myocardial infarction. This efficacy is due to the effects of t-PA and urokinase plasminogen activator (uPA). They are complementary in fibrinolysis so that in combination, their effect is synergistic. Lysis of intact fibrin is initiated by t-PA, and uPA activates the remaining plasminogens. Knockout of the uPA gene, but not the t-PA gene, inhibited fibrinolysis. In the clinic, a minibolus of t-PA followed by an infusion of uPA was administered to 101 patients with acute myocardial infarction; superior infarct artery patency, no reocclusions, and 1% mortality resulted. Endogenous fibrinolysis may provide a paradigm that is relevant for therapeutic fibrinolysis. (J Am Coll Cardiol 2016;68:2099-106) © 2016 Published by Elsevier on behalf of the American College of Cardiology Foundation.

An occlusive intravascular thrombus triggers the cardiovascular diseases that are the leading causes of death and disability worldwide. The only pharmacological means to remove the thrombus is fibrinolysis. There are 2 plasminogen activators in the blood responsible for fibrinolysis: tissue-type plasminogen activator (t-PA) and urokinase plasminogen activator (uPA). They exist in both single-chain and plasmin-cleaved 2-chain forms. With t-PA, both forms have the same activity (1), whereas the single-chain form of uPA is a proenzyme (pro-uPA) and the 2-chain form is an active enzyme (2-chain uPA, the enzymatic form of uPA [tc-uPA]) (2).

The fibrin specificity of t-PA is mediated by high fibrin affinity (3,4), which promotes plasminogen activating activity about a thousand-fold (5). It was the first fibrin-specific plasminogen activator, and became the first recombinant protein on the market (6). The clinical experience with t-PA in acute myocardial infarction (AMI), ischemic stroke, and venous thromboembolism has been extensive. As a result, t-PA became essentially equated with

fibrinolysis, as shown by it frequently not being identified specifically in publications on clinical fibrinolysis.

The anticipated benefits of t-PA were never fulfilled, and it has been largely replaced by percutaneous coronary intervention (PCI) as the treatment of choice for AMI in urban centers in the United States, although it is still used in rural areas too far from a catheterization facility. As pre-treatment, or facilitated PCI, t-PA significantly increased both thrombotic and hemorrhagic complications of PCI (7), and its use has been discouraged. In ischemic stroke, t-PA is used in only about 5% of patients (8), and in venous thromboembolism, a hemorrhagic risk has limited its use to patients with hemodynamic instability due to the emboli (9).

The enzymatic form of uPA, tc-uPA, has been known since the 1940s as a nonspecific activator, like streptokinase (SK), which caused bleeding complications. The single-chain uPA proenzyme, pro-uPA, was not identified and isolated until 1980 (10). It has no fibrin affinity, but is nevertheless fibrin specific (11).



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ABBREVIATIONS AND ACRONYMS

AMI = acute myocardial infarction

ICH = intracranial hemorrhage

PCI = percutaneous coronary intervention

pro-uPA = proenzyme urokinase plasminogen activator

SK = streptokinase

tc-uPA = 2-chain urokinase plasminogen activator, the enzymatic form of uPA

TIMI = Thrombolysis In Myocardial Infarction

t-PA = tissue-type plasminogen activator

uPA = urokinase plasminogen activator

Being a proenzyme, it remains inert in blood until it reaches a clot, a pharmacologically advantageous property. Unfortunately, this property is undermined by dose-dependent plasma instability, because it activates plasma plasminogen at certain therapeutic concentrations, and plasmin converts pro-uPA to tc-uPA. For example, in a study where pro-uPA and SK were compared in AMI, bleeding complications occurred in 14% of the pro-uPA patients, of whom 4% required transfusions and 2 patients had an intracranial hemorrhage (ICH) (12). At physiological and lower therapeutic concentrations, pro-uPA is stable in plasma and is an essential component of the endogenous fibrinolytic system.

FIBRINOLYSIS WITH t-PA

The U.S. Food and Drug Administration approved t-PA for AMI treatment in 1987 but, to show that it reduced mortality compared with SK, additional clinical trials were performed starting in 1990. Three megatrials with an unprecedented total of 95,740 patients were required before a significant p value was reached for a mortality difference (13-15). This was in only 1 of 4 groups in the last trial, in which an accelerated rate of t-PA infusion induced a significant 30-day mortality difference (6.3% vs. 7.0%) (15). Also unexpected was the finding that the ICH rate was significantly higher with t-PA than SK, despite the nonspecific and indirect mode of action of SK. These results did not stand up to a Bayesian analysis, which concluded, “the clinical superiority of t-PA over SK remains uncertain” (16).

As heralded by these trials, t-PA’s limited efficacy—a $16 \pm 10\%$ reocclusion rate (17)—prothrombotic hematologic effects, such as thrombin generation and platelet activation (18-22), and bleeding side effects, such as ICH, led to alternative therapies.

PCI VERSUS t-PA

In a review of 23 PCI trials involving 7,739 patients, 3,867 were randomized to treatment with fibrinolysis (2,939 with t-PA, 928 with SK) and 3,872 patients to primary PCI (23). A significantly better outcome was obtained with PCI for both the short-term and long-term outcomes. The post-treatment Thrombolysis In Myocardial Infarction (TIMI) flow grade 3, an important predictor of good outcome (24,25), was also significantly better with PCI than in the GUSTO (Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries) trial (26).

A special challenge for primary PCI treatment is reducing the time to reperfusion. Reduction in AMI mortality is greatest if reperfusion is accomplished within 1 to 2 h of symptom onset (27), and in patients who received reperfusion treatment within 70 min of symptom onset, the mortality was 1.2% (28). In animal models, the longer the coronary occlusion, the less salvageable myocardium remains (29). Therefore, pre-treatment was tested.

FACILITATED PCI WITH t-PA

Although t-PA pre-treatment doubled the number of patients with initial TIMI flow grade 3 patency, the final TIMI flow grade 3 patency rates did not differ in the 2 groups (89% vs. 88%), consistent with t-PA’s significant rethrombosis rate (17). In addition, more bleeding, reinfarctions, and strokes occurred with facilitated PCI (30). By contrast, with pro-uPA, neither a prothrombotic effect nor reocclusions were found in AMI (31), suggesting that the complications were related to t-PA rather than to fibrinolysis.

It was concluded that t-PA facilitated PCI offered no benefit, an outcome lamented in a commentary in a prominent journal as “Facilitated angioplasty: paradise lost” (32), because there is no alternative to shorten the time to reperfusion.

Interestingly, these studies found that in 15% of patients with ST-segment elevation myocardial infarction, TIMI flow grade 3 was already found at the time of the primary PCI (33), and these patients fared significantly better (24,25). This illustrates the efficacy of endogenous fibrinolysis in which the t-PA concentration is $\geq 1,000$ -fold lower than the therapeutic one.

t-PA FIBRINOLYSIS IN ISCHEMIC STROKE

In ischemic stroke, a t-PA dose reduction was required due to a 20% symptomatic ICH incidence when the usual AMI dose was used (34). As a result, the t-PA recanalization decreased 30% to 40%, making it ineffective for larger thrombi in proximal occlusions (35,36). Even at the reduced dose, the symptomatic ICH rate was 6% to 7% (37).

In the largest trial, 1,500 patients per group were randomized to t-PA or standard care and treated within 6 h. The proportion of patients alive and living independently at 6 months was 37% with t-PA and 35% for the controls. Although insignificant, a secondary ordinal analysis found a significant ($p < 0.001$) favorable shift in the functional score in the t-PA-treated patients, but more patients died with t-PA in the first 7 days (11% vs. 7%; $p = 0.001$) (38). The authors concluded that early treatment with t-PA should be

encouraged, but others were more skeptical, including the American Academy of Emergency Medicine. A 2002 position statement concluded that “evidence regarding the efficacy, safety, and applicability of tPA for acute ischemic stroke is insufficient to warrant its classification as standard of care” (39). Although t-PA is used in only about 5% of patients with ischemic stroke overall (8), it is used in a higher percentage of patients in specialized centers. The potential benefit of t-PA for reperfusion in stroke has been established, but efficacy and safety need to be improved.

More recently, endovascular procedures are being used more frequently, with 3 trials published in the first 2 months of 2015 (40–42). Only patients with proximal thrombi in principal arteries in the anterior circulation are currently eligible.

PULMONARY EMBOLISM, DEEP VEIN THROMBOSIS, AND LIMB ISCHEMIA

PULMONARY EMBOLISM. Because anticoagulants provide effective and safe treatment for the majority of patients, fibrinolysis is currently limited to hemodynamically unstable patients at risk of death (9). In a study of 1,006 such patients randomized to anticoagulants or fibrinolysis with mutant t-PA, fibrinolysis reduced the risk of hemodynamic decompensation and death (2.6% vs. 5.6%), but increased the risk of major hemorrhage (6.3% vs. 1.2%) and stroke (2.4% vs. 0.2%). It was concluded that, “great caution is warranted when considering current fibrinolytic therapy for this indication” (43).

DEEP VEIN THROMBOSIS. In the CaVenT (Catheter-Directed Venous Thrombolysis in Acute Iliofemoral Vein Thrombosis) study of post-thrombotic morbidity, 209 patients with iliofemoral deep vein thrombosis were randomized to catheter-directed fibrinolysis with local infusion of t-PA (20 mg over 24 h) compared with heparin alone. A “relevant” 14.4% reduction of post-thrombotic syndrome was obtained, accompanied by a 21.7% bleeding risk at puncture sites and into the calf, causing compartment syndrome (44). Therefore, a modest efficacy was shown, accompanied by bleeding complications as in the experience described in the preceding text. The largest study, ATTRACT (Acute Venous Thrombosis: Thrombus Removal With Adjunctive Catheter-Directed Thrombolysis), is ongoing.

ACUTE LIMB ISCHEMIA. Despite local delivery of the fibrinolytic treatment, the usual contraindications due to bleeding risk used with systemic fibrinolysis are recommended. Similar results are observed when t-PA (0.5 to 1.0 mg/kg/h) and its mutant forms (reteplase and tenecteplase) or tc-uPA have been used.

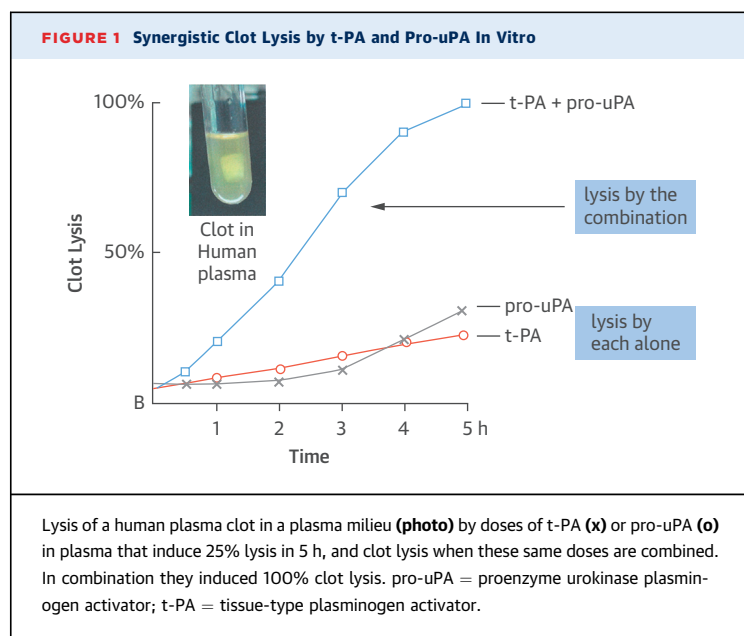
Catheter placement within the thrombus is a prerequisite for this indication, and a positive guidewire test has been found to be a favorable prognostic sign for lysis (45). Successful lysis (>75% thrombus removal) was achieved in 72% of 119 patients; reocclusions occurred within 30 days in 13%, amputations were required in 6%, and life-threatening complications, including bleeding and stroke, occurred in 12% (46). Bleeding complications remain the main deterrent to more widespread use of fibrinolysis for these indications.

LESSONS FROM ENDOGENOUS FIBRINOLYSIS

There is a noteworthy contrast between the limited therapeutic efficacy of t-PA and its efficacy in the endogenous fibrinolytic system, especially because most of the t-PA in blood is in an inactive complex with its inhibitor, plasminogen activator inhibitor-1 (47). Nevertheless, TIMI flow grade 3 patency in 15% of AMI patients (33) was induced, which compares with 45% of patients at 24 h by therapeutic t-PA at a 1,000-fold higher concentration (26). In addition, endogenous fibrinolysis degrades fibrin regularly, as seen by the fibrin degradation product, D-dimer (a fibrinolysis-derived fibrin degradation product), which has a normal plasma concentration of 110 to 250 ng/ml. Only in the presence of an unusually potent autoantibody anticoagulant was the D-dimer level suppressed to 6 to 33 ng/ml (48), establishing that D-dimer in plasma is derived from fibrinolysis of intravascular fibrin, which was suppressed by the anticoagulant.

Therefore, the efficacy of endogenous fibrinolysis is of interest due to its relevance as a model for therapeutic fibrinolysis. The only difference between the 2 systems is that the endogenous system uses t-PA in combination with uPA. Both activators are in blood, and there is a body of evidence that both are required for optimal fibrinolytic efficacy. Moreover, contrary to expectations, uPA, rather than t-PA, was identified as the dominant activator in fibrinolysis.

For example, 2 different gene knockout studies showed that eliminating t-PA had no apparent effect on lysis of an intravascular clot and did not induce fibrin deposition, whereas knocking out uPA caused significant inhibition of clot lysis and some fibrin deposition. Knocking out both genes had a significantly stronger effect, suppressing lysis entirely and causing extensive fibrin deposition (49,50). Therefore, only in the presence of uPA was t-PA's contribution to fibrinolysis evident, and the importance of both activators in combination was also illustrated by the findings.



Second, t-PA and pro-uPA have complementary modes of action, as each activates a different fibrin-bound plasminogen (51,52), and it is fibrin-bound plasmin that is responsible for fibrinolysis. The complementary functions of the activators also make their combined effects synergistic in fibrinolysis (Figure 1) (53), and provide an explanation for the efficiency of endogenous fibrinolysis.

Third, during pro-uPA plasminogen activation, pro-uPA is reciprocally activated by plasmin to the enzyme, tc-uPA (54), which promotes and completes fibrinolysis. Therefore, uPA has 2 fibrinolytic functional states that contribute to fibrinolysis and explain its dominance because t-PA has only 1 functional state (1), consistent with the gene knockout findings (51,52). In addition, this uPA property is also in keeping with a study comparing t-PA and pro-uPA clot lysis rates; that of pro-uPA was consistently twice that of t-PA (55).

Fourth, t-PA and uPA have sequential fibrinolytic functions. At the site of an occlusive thrombus, t-PA stored in the endothelium of the vessel wall is released, and initiates fibrinolysis by binding to its binding site on the D-domain of intact fibrin (3) where plasminogen is also located (4). Fibrin binding of t-PA to this site promotes the kinetics of its activation of this plasminogen by about 1,000-fold (5), initiating fibrinolysis. Fibrin degradation exposes new plasminogen binding sites (56), the first of which is an unusual triple carboxy-terminal lysine-binding site on the fibrin E-domain. This induces a special conformational change in plasminogen, against which pro-uPA's intrinsic activity is promoted 250-fold, enabling pro-uPA to

activate it (57), followed by reciprocal activation of pro-uPA to tc-uPA, as noted (54). The high-affinity substrate binding to this plasminogen is responsible for pro-uPA's fibrin specificity.

Therefore, different fibrin domains promote fibrin-dependent plasminogen activation by t-PA and pro-uPA. This was confirmed in a study in which plasminogen activation was measured using the isolated fibrin domain fragments. Plasminogen activation by t-PA was strongly promoted only by the fibrin fragment D-domain, whereas that by pro-uPA was promoted only by the E-domain (58), reflecting the complementary modes of action of the activators (51). Because t-PA's fibrinolytic function is to initiate fibrinolysis, this could, in time, be initiated by the intrinsic activity of pro-uPA or a little tc-uPA, as previously shown (51), and provides an explanation for why a t-PA knockout had no apparent effect on fibrinolysis (Central Illustration) (49,50).

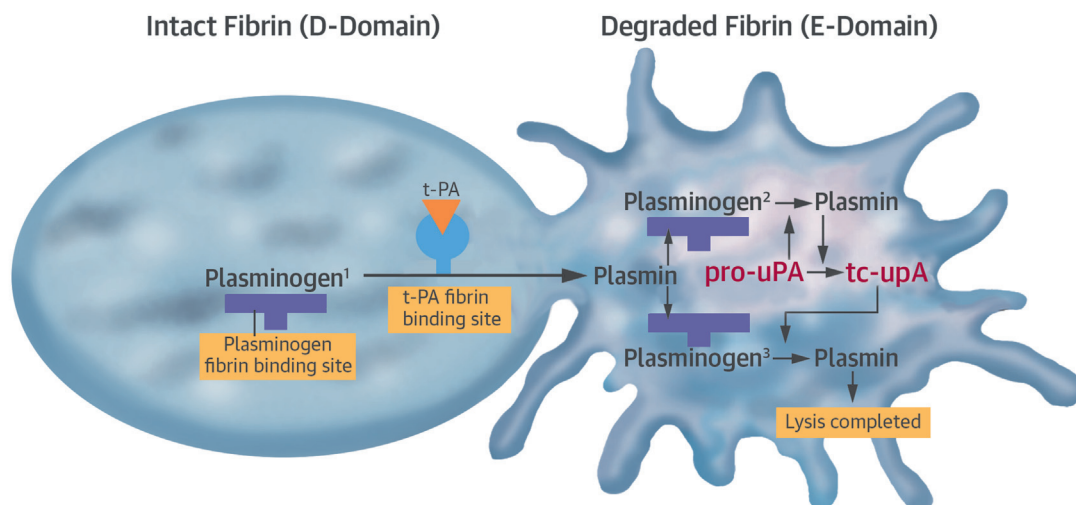
IN VIVO AND CLINICAL TESTING OF t-PA AND PRO-uPA IN COMBINATION

In vitro, a synergistic effect of t-PA and pro-uPA combinations has long been recognized (52,53) (Figure 1) and shown in vivo in animals (59). The combination was subsequently studied clinically in AMI in 2 small studies. In the first, 38 patients with ST-segment elevation myocardial infarction were given 12 mg of t-PA infused for 30 min and 48 mg of pro-uPA infused for 40 min. A TIMI flow grade 3 rate in the infarct artery at 90 min was seen in 57.6% of the patients. It was concluded that the findings "support the potential use of t-PA and pro-uPA in place of monotherapy" (60). In the second study, 23 patients with AMI were given 20 mg of t-PA and 12.3 mg of pro-uPA for 60 min. "Successful infarct artery patency (TIMI flow grade 2 or 3) occurred in 78% of patients" (61). It was concluded that, "combination therapy may allow substantial reductions in dosage" (61). In both of these studies, some bleeding complications occurred.

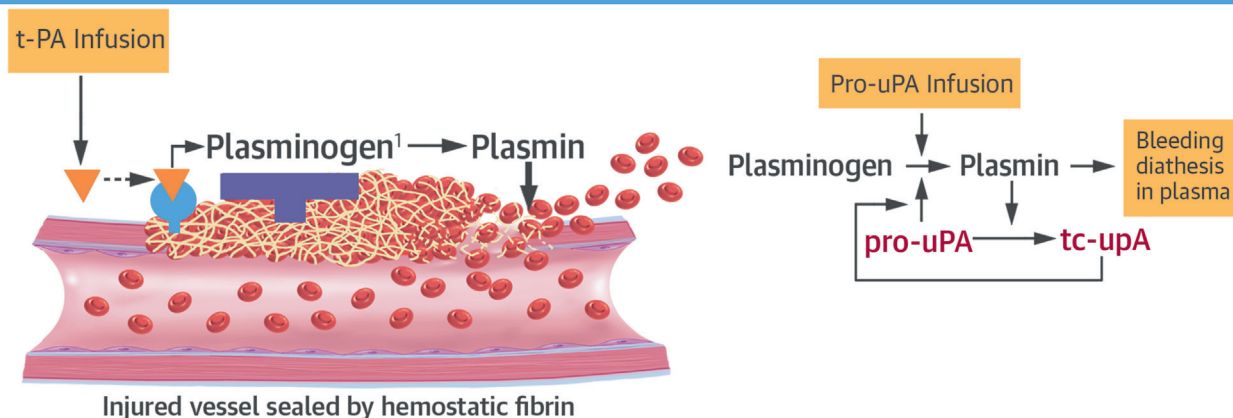
In the largest multicenter study, the PATENT (Pro-Urokinase and t-PA Enhancement of Thrombolysis) trial, 101 patients with ST-segment elevation myocardial infarction received a sequential combination of t-PA and pro-uPA on the basis of their natural sequential functions. In the first 10 patients, a 10-mg bolus of t-PA was administered to initiate fibrinolysis, which was followed by an infusion of 40 mg/h of pro-uPA (50% of the standard AMI infusion rate) for 90 min. This proved to be too much t-PA because it caused some bleeding, as in the 2 studies described. Therefore, the t-PA dose was reduced to

CENTRAL ILLUSTRATION Fibrinolysis and Bleeding by t-PA and pro-uPA

Fibrinolysis by t-PA and u-PA



Bleeding by t-PA and by u-PA



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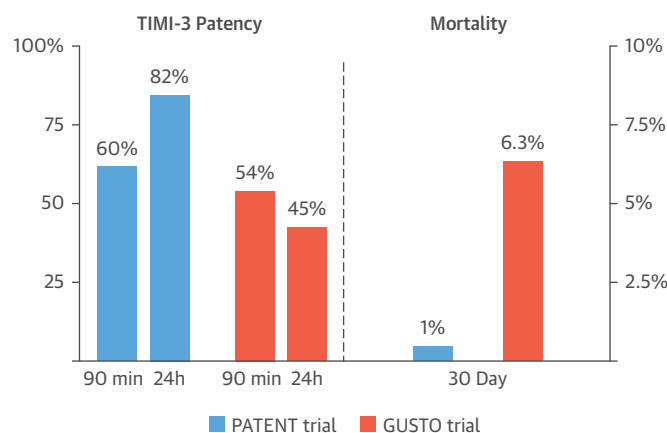
(Top) Fibrinolysis is initiated by t-PA, which binds to its binding site on intact fibrin (D-domain) and activates the fibrin-bound plasminogen at that site to plasmin. This initiates fibrin degradation by plasmin and creates 2 additional plasminogen binding sites, the first of which, on the fibrin E-domain, induces a special plasminogen conformation that enables pro-uPA itself to activate this plasminogen to plasmin (long arrow). Plasmin induces reciprocal activation of pro-uPA to tc-uPA, which then activates the remaining plasminogen and completes fibrinolysis. **(Bottom)** Bleeding: hemostatic fibrin is intact fibrin and, therefore, it contains the same t-PA and plasminogen binding sites that are on the fibrin D-domain shown in the top panel. As a result, hemostatic fibrin is vulnerable to lysis by therapeutic doses of t-PA. High-dose pro-uPA activates plasma plasminogen and plasmin activates pro-uPA, generating more plasmin. pro-uPA = proenzyme urokinase plasminogen activator; tc-uPA = 2-chain urokinase plasminogen activator, the enzymatic form of uPA; t-PA = tissue-type plasminogen activator; uPA = urokinase plasminogen activator.

5 mg (5% of the standard AMI dose) for the remaining 91 patients, with the same pro-uPA infusion, and there were no further bleeding episodes.

A TIMI flow grade 3 patency of the infarct artery at 90 min occurred in 60% of patients increasing to 82% at 24 h. No reocclusions or strokes and only 1 death

occurred in the study (62). These results compare very favorably with the best of the t-PA trials (GUSTO) in which the TIMI flow grade 3 patency at 90 min was 54% and decreased to 45% at 24 h, reflecting t-PA's reocclusion rate; there were 1.5% strokes and 6.3% mortality (Figure 2) (63).

FIGURE 2 TIMI Flow Grade 3 Patency Rates and Mortality in the PATENT and GUSTO Trials



The TIMI flow grade 3 patency rates in the PATENT trial, in which a low-dose sequential combination of t-PA (5 mg) and pro-uPA (40 mg/h) was used (62), and those in GUSTO, the t-PA megatrial in which the best results in AMI were obtained (15). AMI = acute myocardial infarction; GUSTO = Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries; PATENT = Pro-Urokinase and t-PA Enhancement of Thrombolysis; TIMI = Thrombolysis In Myocardial Infarction; other abbreviations as in Figure 1.

MISCONCEPTIONS ABOUT uPA AND THE FIBRINOLYTIC SYSTEM

Certain uPA properties and misconceptions delayed its recognition and contributed to the current state of the art of fibrinolysis. For example, enzymatic uPA, tc-uPA, was known since the 1940s as a non-fibrin-specific activator, like SK, with a high bleeding risk. The proenzyme, pro-uPA, was not identified and isolated until 1980 (63), and because it had no fibrin affinity, recognition of its fibrin-specific fibrinolytic effect was delayed. Moreover, in monotherapy clinical trials, at the concentrations required, pro-uPA in plasma converted to tc-uPA, causing bleeding complications (12).

Pro-uPA is inactivated by the clotting enzyme thrombin (64); thus, its activity was destroyed inadvertently in many fibrinolysis studies in which lysis was preceded by thrombin clotting, giving the

impression that only t-PA was involved in intravascular fibrinolysis. This concept was reinforced by the finding that uPA binds to a cell-surface uPA receptor, and is responsible for extravascular plasminogen activation important for cell migration. It was concluded that uPA was an extravascular plasminogen activator (65), a concept that has tended to persist (66). Much evidence to the contrary has had little effect on this concept, including the finding that a uPA cell-surface receptor knockout had no measurable effect on fibrinolysis by uPA, contrary to expectations (50).

Finally, because most of the uPA is located on the surface of platelets (67,68) and monocytes (69), rather than free in the plasma, it has often gone undetected. All of these pro-uPA properties help to explain why its essential role in fibrinolysis has been overlooked.

CONCLUSIONS

The 2 natural fibrin-dependent plasminogen activators have complementary fibrinolytic functions so that both are needed to complete fibrinolysis. As a result, t-PA and pro-uPA are also synergistic in combination, which provides an explanation for how their low physiological concentrations can induce fibrinolysis. In contrast, fibrinolytic therapy with high-dose t-PA has been inadequate and risky, which has limited its use and resulted in its partial replacement by endovascular procedures for certain indications.

Fibrinolysis, however, remains an essential therapeutic option, being the only means by which rapid, pharmacological removal of a thrombus is possible and reperfusion can be restored at a time when the greatest benefit is achievable. A different fibrinolytic regimen from t-PA monotherapy is required for this objective, and the efficient biological paradigm of endogenous fibrinolysis provides an example that may be difficult to improve upon.

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