Volume 2 • Issue 4 • 2020

Research Article

Journal of Medical Research and Case Reports

Fibrinolysis has been Misunderstood and as a Result its Therapeutic Potential has Thus far been Wasted

Victor Gurewich MD*

Professor of Medicine, Harvard Medical School, 300 Mt Auburn St, Cambridge, MA 02138, Vascular Research Laboratory, Mount Auburn Hospital, Cambridge, MA, USA

***Corresponding Author:** Victor Gurewich, Professor of Medicine, Harvard Medical School, 300 Mt Auburn St, Cambridge, MA 02138, Vascular Research Laboratory, Mount Auburn Hospital, Cambridge, MA, USA.

Received: August 15, 2020; **Published:** August 24, 2020

Abstract

Tissue plasminogen activator (tPA) was approved for therapeutic fibrinolysis in 1987, when it replaced streptokinase (SK) for the treatment of acute myocardial infarction (AMI). It remains the fibrinolytic of choice today although over the last decade fibrinolysis has been replaced by percutaneous coronary intervention (PCI) whenever possible. However, PCI is a technically demanding procedure that is time-consuming whereas for AMI "time is myocardium" and only rapid reperfusion will salvage function of ischemic myocardium. Fibrinolysis is the simplest fastest method, but it requires both plasminogen activators, the second one being urokinase plasminogen activator (uPA), the native form of which is a proenzyme, prouPA. A combination of a 5 mg bolus of tPA followed by a prouPA matches the natural paradigm and was shown to be more effective safer than tPA monotherapy. Activator gene knockout studies also showed that both activators are required, but that uPA rather than tPA was the dominant one, as well as the one neglected for the past 33 years.

Introduction

Common pathologies like AMI and ischemic stroke are usually triggered by a blood clot or thrombus for which therapeutic fibrinolysis with tPA has been a standard treatment. This plasminogen activator was believed responsible for fibrinolysis due to its high fibrin clot affinity [1]. It was developed to replace streptokinase, an indirect, non-specific plasminogen activator of bacterial origin.

Since tPA was of biological origin and was a direct plasminogen activator, it was expected to be much more effective and safer. Three comparative trial were conducted with a total of 95,740 AMI patients [2-4]. In the first two trials, the 30 day mortality with tPA or SK was identical [2, 3]. Only in the third trial did tPA induce a significantly greater mortality reduction but it was only in one of the four groups in this trial [4]. A Bayesian analysis of the three trials reached the conclusion that a significant mortality difference between tPA and SK treatment had not been established [5]. In all three studies, a significantly greater incidence of symptomatic intracranial hemorrhage occurred with tPA, which was also unexpected.

An explanation for these results was never given. Instead, tPA received FDA approval for the treatment of AMI [6], and has remained the fibrinolytic of choice ever since. However, consistent with these early results, the clinical efficacy of tPA in AMI and later in ischemic stroke has been disappointing. As a result, the treatment of choice

for AMI is now percutaneous intervention (PCI), despite its being significantly more time-consuming and costly. An explanation for tPA's poor efficacy can be found in the literature.

tPA monotherapy was based on a misunderstanding of fibrinolysis

There are two biological plasminogen activators, tPA and uPA whose properties are not only different but highly complementary [9]. tPA is an enzyme that strongly promoted by fibrin [1] to which it has a high affinity [10]. By contrast, uPA is a proenzyme, prouPA, with no fibrin-affinity but nevertheless has a fibrin-specific mode of action [11]. This is was found to be due to a strong substrate affinity related to a conformational change in plasminogen [12]. Due to their complementarity, the combination of the activators induced a synergistic effect [9], especially when the combination was sequential starting with tPA . The synergistic effect enables lower, safer doses to be used.

Despite this published evidence, tPA was evidently too well-established for it to be put into question. Instead, it was believed that uPA was principally an extravascular plasminogen activator [13].

Gene knockout animal studies contradicted this concept

Two independent gene deletion studies showed that fibrinolysis requires both plasminogen activators. When the tPA gene was deleted, it had surprisingly little effect on the rate of spontaneous lysis of an intravascular clot formed in the animals. By contrast, a uPA knockout induced significant inhibition of clot lysis and caused some spontaneous fibrin deposition. Knocking out both genes arrested clot lysis completely and induced extensive fibrin deposition in the animals. The authors concluded that both activators were required for fibrinolysis to function normally, and that uPA was the dominant activator [22, 23]. These findings and conclusions are consistent with in vitro and in vivo clot lysis studies [24].

The Physiological Mechanism of Fibrinolysis

Endogenous fibrinolysis is an ongoing process as evidenced by the invariable presence of the fibrinolytic degradation product, D-dimer in blood (normal concentration: 110-250 ng/ml) Only in the presence of a potent thrombin autoantibody that inhibited fibrin formation has a D-dimer closer to zero (6-33 ng/ml) been reported [7]. Interestingly, this endogenous fibrinolysis is free of bleeding complications, in contrast to therapeutic fibrinolysis. Nevertheless, it is relatively potent since in 15% of untreated AMI patients complete patency (TIMI-3) of the infarct artery was found at the time of the initial catheterization [8]. This endogenous lysis rate compares with a 45% rate in patients treated with full dose tPA in whom the blood tPA concentration is almost 1,000-fold higher. Since the endogenous plasma concentration of tPA is only ~5 ng/ml, it is not possible that this degree of lysis is due to tPA alone.

In the endogenous system, tPA's function is limited to the initiation of fibrinolysis, a function analogous to that of the starter in a car. This occurs when tPA is released from the endothelium of the vessel wall at the site of an intravascular thrombus. Due to its high fibrin affinity, tPA binds to the thrombus [13] at a fibrin site proximal to plasminogen bound to lysine Aα157 on the fibrin surface [14]. This tPA, plasminogen, fibrin ternary complex promotes tPA's plasminogen activation about 1,000-fold [15] and initiates fibrinolysis.

Since tPA has no second high affinity fibrin binding site and is otherwise a weak plasminogen activator [16], this completes tPA's function in fibrinolysis. It also explains tPA's high dose requirements when it is used alone since to activate the remaining two fibrin-bound plasminogens high doses are necessary, but even these are not fully effective.

The initiation of fibrin degradation by tPA creates new plasminogen binding sites on fibrin [18] which are two in number [17]. These plasminogens are more efficiently activated by prouPA, explaining why the activators have complementary modes of action and are synergistic in fibrinolysis when combined [18], especially when combined sequentially starting with tPA [19].

A Therapeutic Test of the Sequential Activator Combination

The PATENT study was a multi-center trial in 101 consecutive patients with AMI treated with a mini bolus of tPA (10 mg in ten and 5 mg in the remaining 91 patients) followed by a 90 minute infusion of prouPA (40 mg/h). This fibrinolytic sequential combination induced a TIMI-3 patency at 24 h in 82% of the patients and a 30-day mortality of 1% [25]. These results compared very favorably with GUSTO, the best of the tPA mega trials in which the TIMI-3 infarct artery patency at 24 h was only 45%, and the mortality was 6% [26]. These clinical results were consistent with in vitro clot lysis data showing that together the activators have a synergistic fibrinolytic effect [9] especially when they are in a sequential combination [19], an effect also reported in an animal study [27], and cited in another review [24].

Platelets Bind prouPA and Extend its Half-Life

In the PATENT trial it was noted that about 30% of the lysis took place well after the end of the prouPA's infusion [18]. Since the prouPA's plasma half-life is only about 7 minutes, this required an explanation

In a study of the prouPA intrinsic to blood, about 20% of it was found bound to the outer platelet membrane [28] and evidence of a novel uPA binding protein on the membrane was found [29]. Platelets also were shown to promote fibrinolysis by prouPA [30]. Since platelets have a half-life of 2.5 days, the platelet-bound prouPA has a greatly prolonged fibrinolytic half-life. This property may also help explain the low rate of reocclusion reported with prouPA fibrinolysis [25, 31].

Fibrinolysis and Bleeding

Hemorrhagic side effects, especially intracranial hemorrhage (ICH), have limited utilization of fibrinolytic therapy. For example, in ischemic stroke a 7% incidence of symptomatic ICH has been reported with tPA treatment [32]. This bleeding risk is specific for tPA, but not with uPA since it does not bind to fibrin. Bleeding by tPA is predominantly due to the lysis of hemostatic fibrin [33] to which tPA binds as it does to all fibrin, especially if it administered as a prolonged infusion. Hemostatic fibrin can be found at vascular repair sites and seems to regularly present judging by the fibrin degradation product D-dimer that is invariably present in blood (110-250 ng/ml). Bleeding from these sites is an unpredictable risk, especially when tPA is administered as an infusion rather than a bolus.

Conclusions

tPA and prouPA, the two natural plasminogen activators, induce fibrinolysis sequentially by complementary modes of action. As a result, when they are administered in a sequential combination, fibrinolytic efficacy is significantly promoted without any loss of fibrin specificity or safety. This natural fibrinolytic regimen has been misunderstood and monotherapy discredited the fastest treatment of thrombotic occlusions available. In a single clinical trial, this paradigm was once used therapeutically in 101 AMI patients. The result was a six-fold reduction in mortality and an almost two-fold increase in coronary patency compared with the best of the tPA mega trials. A second trial in AMI is currently underway, and a trial in ischemic stroke is nearing completion.

References

1. Rijken DC, Hoylaerts M, Collen D. (1982). Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator. J Biol Chem; 257:2920-2925.
2. Gruppo Italiano Per Lo Studio Della Sopravvivenza Nell'Infarto Miocardico. (1990). GISSI-2: A factorial randomised trial of alteplase versus streptokinase and heparin versus no heparin among 12,490 patients with acute myocardial infarction. Lancet 336:65-71.
3. ISIS-3 Third International Study of Infarct Survival Collaborative Group. (1992). 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 339:65-71.
4. The GUSTO Investigators. (1993). An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. N Eng J Med 329: 673-682.
5. Brophy JM, Joseph L. (1995). Placing trials in context using Bayesian analysis. GUSTO revisited by Reverend Bayes. JAMA 273:871-875.
6. Pennica D, Holmes WE, Kohr WJ, Harkins RN, Vehar GA, Wad XCA, Bennett WF, Yelverton, E, Seeburg PH, Heyneker HL, Goeddel DV, Collen D. (1983). Cloning and expression of human tissue-type plasminogen activator cDNA in E.coli. Nature 301:214-221.
7. Baglin TP, Landown R, Frasson R, Huntington JA. (2016). Discovery and characterization of an antibody directed against exosite 1 of thrombin. J Thromb Haemost 14:137-142.
8. Assessment of the Safety and Efficacy of a New Treatment Strategy with Percutaneous Coronary Intervention (ASSENT-4 PCI) investigators. (2006). Primary versus tenecteplase-facilitated percutaneous coronary intervention in patients with ST-segment elevation acute myocardial infarction (ASSENT-4 PCI): randomised trial. Lancet 367:569-578.
9. Pannell R, Black J, Gurewich V. (1988). The complementary modes of action of tissue plasminogen activator (t-PA) and pro-urokinase (pro-UK) by which their synergistic effect on clot lysis may be explained. J Clin Invest 81:853-859.
10. Husain SS, Lipinski B, Gurewich V. (1981). Rapid purification of high affinity plasminogen activator from human plasma by specific adsorption on fibrin-celite. Proc Nat Acad Sci (USA) 78:4265-4269.

11. Gurewich V, Pannell R, Louie S, Kelley P, Suddith RL, Greenlee R. (1984). Effective and fibrin-specific clot lysis by a zymogen precursor form of urokinase (pro-urokinase). A study in vitro and in two animal species. J Clin Invest 73:1731-1739.
12. Liu J, Gurewich V. (1992). Fragment E-2 from fibrin substantially enhances pro-urokinase-induced glu-plasminogen activation. A kinetic study using a plasmin-resistant mutant pro-urokinase (Ala-158-rpro-UK). Biochemistry 31:6311-6317.
13. Collen D. (1999). The plasminogen (fibrinolytic) system. Thromb Haemost 82:259-270.
14. Voskuilen M, Vermond A, Veeneman GH, van Boom JH, Klasen EA, Zegers ND, Nieuwenhuizen W. (1987). Fibrinogen lysine residue Aα157 plays a crucial role in the fibrin-induced acceleration of plasminogen activation, catalyzed by tissue-type plasminogen activator. J Biol Chem 262(13); 5944-5946.
15. Hoylaerts M, Rijken DC, Lijnen HR, Collen D. (1982). Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. J Biol Chem 257: 2912-2919.
16. Rijken DC, Hoylaerts M, Collen D. (1982). Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator. J Biol Chem 257:2920-2925.
17. Suenson E, Lützen O, Thorsen S. (1984). Initial plasmin-degradation of fibrin as the basis of a positive feed-back mechanism in fibrinolysis. Eur J Biochem 140: 513-522.
18. Pannell R, Li S, Gurewich V. (2017). Fibrin-specific and effective clot lysis requires both plasminogen activators and for them to be in a sequential rather than simultaneous combination. J Thromb Thrombolysis 44:210-215.
19. Pannell R, Li S, Gurewich V. (2015). Highly effective fibrinolysis by a sequential synergistic combination of mini-dose tPA plus low-dose mutant proUK. PLOS One 10:1-15.
20. Harpel PC, Chang TS, Verderber E. (1985). Tissue plasminogen activator and urokinase mediate the binding of Glu-plasminogen to plasma fibrin I. Evidence for new binding sites in plasmin-degraded fibrin I. J Biol Chem 260(7):4432-4440.
21. Petersen LC. (1997). Kinetics of reciprocal pro-urokinase/plasminogen activation. Stimulation by a template formed by the urokinase receptor bound to poly (D-lysine). Eur J Biochem 245:316-323.
22. Bugge TH, Flick MJ, Danton MJS, Daugherty CC, Rømer J, Danø K, Carmeliet P, Collen D, Degen JL. (1996). Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. Proc Natl Acad Sci USA 93:5899-5904.
23. Singh I, Burnand KG, Collins M, Luttun A, Collen D, Boelhouwer B, Smith A. (2003). Failure of thrombus to resolve in urokinase-type plasminogen activator gene-knockout mice: rescue by normal bone marrow-derived cells. Circulation 107:869-875.
24. Gurewich V. (2016). Therapeutic fibrinolysis. How efficacy and safety can be improved. J Am Coll Cardiol 68:2099-2106.
25. Zarich SW, Kowalchuk GJ, Weaver WD, Loscalzo J, Sassower M, Manzo K, Byrnes C, Muller JE, Gurewich V for the PATENT Study Group. (1995). Sequential combination thrombolytic therapy for acute myocardial infarction: results of the pro-urokinase and t-PA enhancement of thrombolysis (PATENT) trial. J Am Coll Cardio 26:374-379.
26. GUSTO Angiographic Investigators. (1993). The effects of tissue plasminogen activator, streptokinase, or both on coronary-artery patency, ventricular function, and survival after acute myocardial infarction. N Engl J Med 329:1615-1622.
27. Collen D, Stassen JM, Stump DC, Verstraete M. (1986). Synergism of thrombolytic agents in vivo. Circulation 74:838-842.
28. Park S, Harker LA, Marzec UM, Levin EG. (1989). Demonstration of single chain urokinase-type plasminogen activator on human platelet membrane. Blood 6:1421-1425.
29. Jiang YP, Pannell R, Liu JN, Gurewich V: (1996). Evidence for a novel binding protein for urokinase-type plasminogen activator in platelet membranes. Blood 87:2775-2781.
30. Baeten KM, Richard MC, Kanse SM, Mutch NJ, Degens JL, Booth NA. (2010). Activation of single-chain urokinase-type plasminogen activator by platelet-associated plasminogen: a mechanism for stimulation of fibrinolysis by platelets. J Thromb Haemost 8:1313-1322.
31. Weaver WD, Hartmann JR, Anderson JL, Reddy PS, Sobolski JC, Sasahara A for the Prourokinase Study Group. (1994). New recombinant glycosylated prourokinase for treatment of patients with acute myocardial infarction. J Am Coll Cardiol 241:1242-1248.
32. The IST-3 Collaborative Group. (2012). The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. Lancet 379:2352-2363.
33. Montoney M, Gardell SJ, Marder VJ. (1995). Comparison of the bleeding potential of vampire bat salivary plasminogen activator versus tissue plasminogen activator in an experimental rabbit model. Circulation91:1540-1544.

34. Vermeer F, Bösl I, Meyer J, Bär F, Charbonnier B, Windeler J, Barth H.(1999). Saruplase is a safe and effective thrombolytic agent; observations in 1698 patients: Results of the PASS Study. J Thromb Thrombolys 8: 143-150.