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REVIEW ARTICLE

# Fibrinolysis: a Misunderstood Natural Defense Whose Therapeutic Potential Is Unknown

Victor Gurewich [1]

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## Abstract

Ever since tissue plasminogen activator (tPA) was approved for therapeutic fibrinolysis in 1987, it has been the fibrinolytic of choice. At the same time, it is also recognized that tPA never lived up to its clinical expectations and has more recently been replaced by percutaneous coronary intervention (PCI) as the treatment of choice for acute myocardial infarction (AMI). For other occlusive vascular diseases and for patients in remote areas, tPA remains an essential option. In view of the continued importance of fibrinolysis, it is disappointing that fibrinolysis never evolved beyond what it was when tPA replaced streptokinase (SK) 32 years ago. The endovascular procedure replacement for AMI treatment suffers from being technically demanding, time-consuming, and costly. An untested alternative fibrinolytic paradigm is that of the endogenous, physiological system, which is initiated by tPA but then is followed by the other natural plasminogen activator, urokinase plasminogen activator (uPA). In this combination, it is uPA rather than tPA that has the dominant function. This is also evident from gene knockout studies where deletion of uPA that it has the dominant effect. The fibrinolytic properties of tPA and uPA are complementary so that their combined effect is synergistic, especially when they are administered sequentially starting with tPA. Endogenous fibrinolysis functions without bleeding side effects and is ongoing. This is evidenced by the invariable presence in blood of the fibrin degradation product, D-dimer (normal concentration 110–250 ng/ml). This activator combination, consisting of a mini bolus of tPA followed by a 90-min proUK infusion, was once used to treat 101 AMI patients. Compared with the best of the tPA mega trials, this regimen resulted in an almost a doubling of the infarct artery patency rate and reduced mortality sixfold. To date, a second trial has not yet been done.

**Keywords** Thrombosis · Fibrinolysis · Tissue plasminogen activator · Urokinase plasminogen activator

## Introduction

Common pathologies like AMI and ischemic stroke are usually caused by an intravascular thrombus, for which therapeutic fibrinolysis has been a standard treatment. The plasminogen activator believed responsible for fibrinolysis is tPA, a direct plasminogen activator with a high fibrin clot affinity [1]. It was developed to replace the non-specific plasminogen activator, streptokinase (SK), an indirect activator which as a consequence has a less efficient mechanism of action.

Three trials of unprecedented size comparing mortality between tPA and SK were conducted in a total of 95,740 AMI patients [2–4]. Surprisingly, in the first two trials, no mortality difference was found [2, 3], and in the third trial, tPA induced a small mortality reduction in one of four groups [4]. In all three studies, significantly more symptomatic intracranial hemorrhage occurred with tPA. Bayesian analysis of these findings reached the conclusion that a significant mortality difference between tPA and SK treatment had not been established [5].

Even though these results were paradoxical, they failed to cast any doubt on the conviction that tPA alone was responsible for fibrinolysis. Promptly after the trials, tPA was given approval for therapeutic fibrinolysis for AMI, became the second recombinant protein on the market [6], and has remained the fibrinolytic of choice ever since.

The clinical record of fibrinolysis by tPA alone for AMI and stroke has been consistent with the disapointing results of these early mega trials as also reflected by the increasing use of endovascular procedures as the primary treatment for

✉ Victor Gurewich
vgurewich@tsillc.net

[1] Vascular Research Laboratory, Mount Auburn Hospital, Harvard Medical School, 300 Mt Auburn St, Cambridge, MA 02138, USA

thrombotic occlusions. However, before fibrinolysis as a therapeutic modality is dismissed, the hypothesis that tPA and fibrinolysis are equivalent should be put into question.

The main impetus for this is that the natural, endogenous, fibrinolytic system does not function with tPA alone. It utilizes a sequential combination of tPA and uPA in which the latter not the former is the dominant activator. The endogenous concentration of tPA is only ~ 5 ng/ml in plasma, which also contains a potent tPA inhibitor, plasminogen activator inhibitor-1 (PAI-1). Nevertheless, fibrinolysis is an ongoing process as shown by the generation of the fibrin degradation product D-dimer (normal concentration 110–250 ng/ml). In the presence of thromboembolism, the D-dimer concentration can go up tenfold or more and is used as a sensitive diagnostic test. Only in the presence of a potent thrombin autoantibody inhibiting fibrin formation has a D-dimer level closer to zero (6–33 ng/ml) been reported [7].

Another example of endogenous lysis is from untreated AMI patients in whom spontaneous intracoronary lysis (TIMI-3) was found in 15% of patients at the time of the initial cardiac catheterization [8]. This degree of endogenous lysis compares with a 45% coronary patency rate at 24 h after a full therapeutic dose of tPA [8], at which its blood concentration is about 1000-fold higher, inducing lysis only threefold higher.

It seems evident that this degree of endogeous fibrinolytic activity cannot be explained by an effect of tPA alone.

## tPA Monotherapy Was Based on a Misunderstanding of Fibrinolysis

It is well known that there are two biological plasminogen activators, tPA and urokinase plasminogen activator (uPA), whose properties are different and complementary [9]. tPA is an enzymatic plasminogen with an activity strongly promoted by fibrin [1] to which it also has a high affinity [10]. By contrast, uPA is a proenzyme (proUK) with no fibrin affinity but with a fibrin-specific mode of action [11] due to substrate affinity related to a plasminogen conformational change [12] (Fig. 1).

At the time that tPA was given clinical approval for AMI in 1987, it was already known that tPA and proUK had properties that were complementary [9] and thereby made their combined effects synergistic [11]. However, these findings were eclipsed by the widespread conviction that tPA and fibrinolysis were equivalent and uPA an extravascular plasminogen activator [13].

## The Physiology of Fibrinolysis

In contrast to fibrinolytic therapy, endogenous physiological fibrinolysis utilizes both plasminogen activators, starting with tPA. The tPA is stored in the endothelium of the vessel wall from where it is released at the site of a fibrin thrombus. Due to its high fibrin affinity, tPA binds to fibrin at its binding site on the thrombus [13]. This site is close to the intact fibrin-bound plasminogen on lysine Aα157 [14] forming a ternary complex. This fibrin-bound complex promotes tPA activation of plasminogen about 1000-fold [15] and initiates fibrinolysis.

Since tPA has no second high affinity binding site, this completes tPA's fibrinolytic function, since in the absence of fibrin promotion, tPA is a very weak plasminogen activator [1]. This can also be the cause of tPA's high dose requirements when it is used alone for therapy. Since fibrinolysis involves the activation of three fibrin-bound plasminogens [16] and only one is in the ternary complex with tPA, the other two can only be activated by tPA at very high doses (Fig. 1).

More recently, it was shown that for fibrinolysis to be more effective and fibrin-specific, not only are both plasminogen activators required [17] but also this combination is even more effective when tPA and proUK are administered sequentially starting with tPA [18]. This also corresponds to the endogenous fibrinolytic paradigm. The initiation of fibrin degradation creates new plasminogen-binding sites on the fibrin surface [19], which are two in number [16] and are carboxy-terminal lysines.

The first of these new binding sites is an unusual triple carboxy-terminal lysine site on the fibrin E-2 domain, which induces a conformational change in the plasminogen that binds to it. Against this conformation, the intrinsic activity of proUK increases substantially and becoming equivalent to UK without activation, so that proUK itself is able to activate this plasminogen [12]. This activation is followed by the reciprocal activation of proUK by plasmin [20] and UK then completes fibrinolysis by activating the third plasminogen (see Fig. 1).

In summary, physiological fibrinolysis involves the sequential activation of three fibrin-bound plasminogens [16]. The first is activated by tPA when it binds to intact fibrin and forms a ternary complex [1, 13]. The second is activated by proUK due to a conformational change in plasminogen [12], and the third plasminogen is activated by UK [20]. Therefore, uPA is responsible for activating two out of the three plasminogens and is the dominant activator [18], when it had not even been considered involved.

## Gene Knockout Studies Also Show Both Activators to be Required and for uPA to be Dominant

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

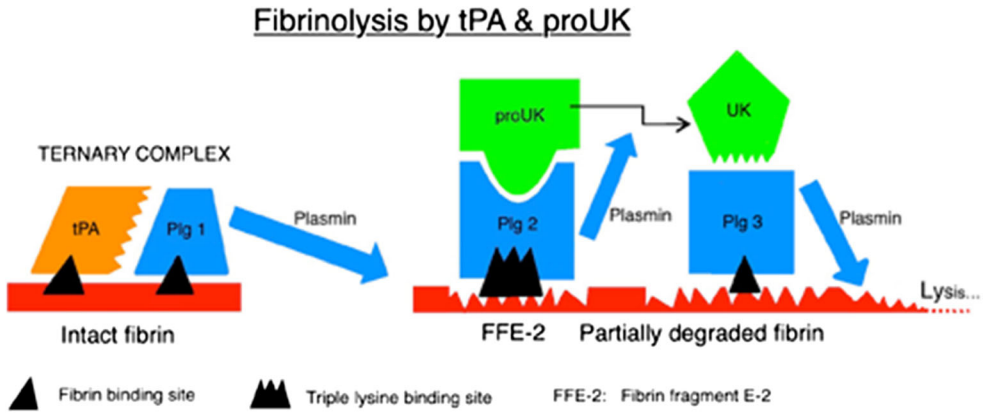


**Fig. 1** Fibrinolysis is initiated when tPA binds to its binding site black triangle on intact fibrin which is adjacent to a plasminogen-binding site, thereby forming a ternary complex with this plasminogen on fibrin. This complex promotes plasminogen activation by tPA 1000-fold [15]. Plasmin then initiates fibrin degradation and this creates two additional plasminogen-binding sites on fibrin (back). The first of these is a triple carboxy-terminal lysine site that induces a conformational change in plasminogen 2 (represented by convex shape). This conformation allows the intrinsic activity of the proenzyme, proUK, to activate it, which is followed by reciprocal activation of proUK by plasmin. UK then activates p3, completing fibrinolysis

authors concluded that both activators were required for fibrinolysis to function normally and that uPA was the dominant activator [21, 22]. These findings and conclusions are consistent with in vitro and in vivo clot lysis studies [23].

## 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-min infusion of proUK (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% [24]. These results compared 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% [25]. The clinical results are 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 [18], an effect also reported in an animal study [26] and cited in another review [23].

This fibrinolytic mechanism is analogous to a car engine, in which tPA is like the starter and uPA the fuel. As in the car, the two components are independent but interdependent and their mode of action is sequential.

## Platelets Bind proUK 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 proUK infusion [17]. Since the proUK plasma half-life is only about 7 min, this required an explanation

In a study of the proUK intrinsic to blood, about 20% of it was found bound to the outer platelet membrane [27] and evidence of a novel uPA binding protein on the membrane was found [28]. Platelets have also been shown to promote fibrinolysis by proUK [29]. Since platelets have a half-life of 2.5 days, binding greatly prolongs the fibrinolytic half-life of this fraction of proUK. Platelet binding is property unique to proUK, specifically its A-chain, since low molecular weight UK did not bind (Fig. 2). This property may also help explain the low rate of reocclusion reported with proUK fibrinolysis [24, 30].

## Fibrinolysis and Bleeding

Hemorrhagic side effects, especially intracranial hemorrhage (ICH), have limited utilization of fibrinolytic therapy. For

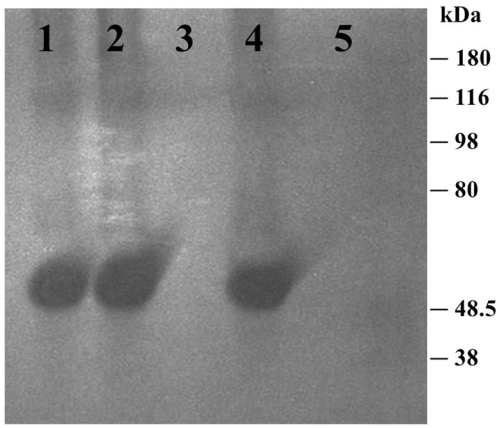


**Fig. 2** Zymogram of platelets after incubation (15 min) with equimolar concentrations (20 nM) of (1) prouPA; (2) prouPA + low molecular weight (LMW) uPA; (3) LMW-uPA; (4) prouPA + tPA; and (5) tPA. Only prouPA bound to platelets. Neither LMW-uPA nor tPA bound under these conditions

example, in ischemic stroke, a 7% incidence of symptomatic ICH has been reported with tPA treatment [31]. This bleeding risk is specific for tPA, consistent with the bleeding etiologies of the two activators being as different as their fibrinolytic mechanisms. This difference has been given little attention but is of practical importance.

Bleeding by tPA is predominantly due to the lysis of hemostatic fibrin [32] to which tPA binds as it does to all fibrin, especially if it is administered as a prolonged infusion. Hemostatic fibrin can be found ay vascular repair sites and seems to be 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 ever-present unpredictable risk with tPA infusion.

By contrast, bleeding by proUK is unrelated to hemostatic fibrin since it does not bind to fibrin and also only activates plasminogen on partially degraded fibrin [23]. Bleeding is due to proUK activation to UK, which can cause plasminemia and degradation of coagulation factors, but this bleeding risk has the advantage of being easily recognized and correctible.

The risk of UK conversion can be minimized by reducing the fibrinolytic dose required, as by using the sequential activator combination which uses only a bolus of 5% of the current standard tPA therapeutic dose [24], and 50% of the proUK infusion required in the proUK monotherapy trials [33].

## Conclusions

tPA and proUK, the two natural plasminogen activators, induce fibrinolysis sequentially by complementary and interdependent 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 resulted in a discrediting of 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 sixfold reduction in mortality and an almost twofold increase in coronary patency compared with the best of the tPA mega trials. A second trial has thus far not been done.

Key Findings

- Common pathologies like stroke and heart attack are usually caused by a thrombus.
- Optimal salvage of function and of life requires restoration of perfusion within 1–2 h, which is most readily accomplished by fibrinolysis.
- However, fibrinolysis with tPA has been inadequate and risky.
- Physiological fibrinolysis utilizes both tPA and uPA and is effective in the absence of bleeding side effects.
- Therefore, it provides a model to make fibrinolytic therapy safer.

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## Compliance with Ethical Standards

**Conflict of Interest** Victor Gurewich MD is the scientific director of Thrombolytic Science International (TSI), a small company that is developing a prouPA mutant for fibrinolytic therapy. Ralph Pannell PhD has no disclosures or conflicts of interest to report.

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