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Review Article

Fibrinolytic Therapy Failed Because of a Misunderstanding; Tpa Must be Combined with Upa as in Nature

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Abstract

In acute myocardial infarction (AMI), reperfusion of the infarct artery within the first two hours is the principal determinant of infarct size and mortality. Only fibrinolytic therapy can meet this reperfusion time requirement. However, tissue plasminogen activator (tPA) has been so ineffective and hazardous that it has been replaced by percutaneous coronary intervention (PCI) as the treatment of choice for AMI, which is a time-consuming treatment. For other indications like ischemic stroke, tPA remains the principal available option. Published studies of fibrinolysis show that in natural, physiological fibrinolysis, tPA initiates fibrinolysis which is then completed by urokinase-type plasminogen activator (uPA), the native form of which is a proenzyme, prouPA. tPA and prouPA have complementary modes of action and in combination their fibrinolytic effect is synergistic. This activator combination is, therefore, more effective than either activator is alone, and the combination is also much safer since significantly lower doses are required. tPA and uPA gene deletion studies in mice confirmed that both activators were required for efficacy, and that uPA was the dominant activator. Superior efficacy by the combination was also seen in clot lysis studies in human plasma. Finally, a clinical proof of this concept has already been done. In a multicenter AMI trial, 101 patients were treated with a mini-bolus of tPA followed by a prouPA infusion. This resulted in almost a doubling of the 24h infarct artery patency rate compared to that in the best of the tPA trials. There were no re-occlusions and the mortality was only 1%, which compares with a 6.3% mortality with tPA.

Introduction

The science historian Thomas Kuhn observed that science tends not to progress as a linear accumulation of new knowledge, but rather undergoes periodic revolutions which he called paradigm shifts [1]. He found that these paradigm shifts were required for new findings that challenge established ones to be accepted. This observation is particularly germane to fibrinolysis in which a therapeutic regimen based on a flawed premise resulted in fibrinolytic therapy becoming discredited and replaced by primary percutaneous coronary intervention (PCI) as the treatment of choice in acute myocardial infarction (AMI). Since PCI is a hospital-based, invasive procedure, coronary reperfusion is inevitably delayed which compromises salvage of myocardium and mortality reduction.

In non-human primates, the duration of coronary occlusion is the main determinant of myocardial infarct size [2]. Similarly, in AMI when coronary reperfusion takes place within 2 hours of the event, infarct size was 2% by scan whereas the figure jumped to 12% at 3 hours. After 4 hours, little further cardiac damage occurred [3]. Similarly, in a study of primary coronary angioplasty, it was found that pre-procedural patency (TIMI-3) rather than post PCI patency was an independent predictor of one year survival [4]. Finally, in higher risk AMI patients, the 6 month mortality increased from 5% to 13% when reperfusion was delayed from less than 2 to 4 hours or more [5]. All these findings emphasize the importance of giving a high priority to providing as rapid reperfusion as possible. .

Coronary reperfusion within the limited time-period required for an optimal clinical outcome can be can only be achieved with fibrinolysis for most patients. However, this is not possible to do with tPA alone as comparative studies with PCI have established. Even when PA was used for pretreatment, as in facilitated PCI, tPA had to be abandoned because it increased the bleeding and rethrombosis complication rate significantly [6].

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Background

The tPA properties of high fibrin-affinity and fibrin-dependent plasminogen activation made it a compelling choice for fibrinolytic therapy, especially since only streptokinase (SK) and two-chain (tc) uPA, were available at the time. These activators induced bleeding side-effects due fibrin-independent plasminogen activation, a mechanism which also limited their efficacy. By comparison, tPA was expected to be both more effective and safer.

Surprisingly, three mega-trials comparing tPA with SK in a total of more than 95,000 AMI patients [7-9] showed that the death rate with tPA was not significantly different from that with SK [10]. Moreover, tPA caused significantly more intracranial hemorrhage than SK [8,9]. These results were paradoxical in light of their very different properties, but no explanation for this paradox was ever offered. Had this occurred, it might have raised the question of whether the assumption that tPA was responsible for endogenous fibrinolysis was still tenable.

As it was, tPA was given regulatory approval for AMI treatment after these trials in 1987 and it has remained the fibrinolytic of choice. Over the last decade, due to its continued limited efficacy and bleeding risk, tPA has been replaced by primary PCI as the treatment of choice in AMI, and endovascular procedures are also being used increasingly in ischemic stroke.

The adoption of PCI solved the problem of tPA bleeding complications, but since it is a technically demanding, hospital-based procedure, it makes optimal reperfusion within two-hours of the event out of reach for most patients. For this a simpler, pre-hospitalization reperfusion method is required, meaning fibrinolysis.

To make fibrinolysis more successful, both its efficacy and safety must be improved over that of tPA. To find such a new fibrinolytic, a useful starting point is the endogenous fibrinolytic system, since it functions with remarkable efficiency.

Fibrinolysis in the Endogenous Physiological System

The standard therapeutic dose of tPA in AMI is 100 mg infused over 90 minutes, giving a plasma concentration of about 3-4 µg/ml [9]. By comparison, the endogenous tPA plasma concentration is 10-12 ng/ml, of which much of it is in an inactive complex with its plasma inhibitor, plasminogen activator inhibitor-1 (PAI-1) [11]. Therefore, the endogenous tPA concentration is about 1,000-fold lower than the therapeutic one, but it nevertheless generates the products of fibrinolysis continuously.

This is evidenced by the invariable presence in plasma of the fibrin degradation product D-dimer, the normal concentration of which is 112-250 ng/mL. Since D-dimer represents 60% of the fibrin monomer mass, this concentration represents a steady state degradation of fibrin of about 1 mg in 3,000 ml of plasma. Only in a patient with a potent anticoagulant, an autoantibody to thrombin, was the D-dimer plasma concentration reported to be closer to zero (12-32 ng/ml) [12]. In the presence of venous thromboembolism, the D-dimer concentration increases as much as 30-fold, representing degradation of a corresponding amount of fibrin by endogenous fibrinolysis showing that the endogenous system has considerable reserve.

This level of fibrinolytic activity cannot be due to an effect by the small amount of endogenous free tPA alone, implicating the presence of an additional activator.

Physiological Fibrinolysis Requires Two Plasminogen Activators

Endogenous fibrin formation is required not only for hemostasis, but also for the ongoing repair of wear and tear injuries in the vasculature. There are two plasminogen activators in blood to keep this fibrin formation from blocking blood flow. The second activator is uPA, the native form of which is the proenzyme, prouPA. Therefore, in contrast to tPA, uPA has two forms, a single-chain proenzyme and a two-chain enzyme (tcuPA). During fibrinolysis, prouPA is activated to tcuPA. .

Since most of the prouPA in blood is carried on the surface of platelets [13,14] and monocytes [15], rather than being in the plasma like tPA, it has often escaped detection. Moreover, prouPA lacks fibrin affinity but has a cell surface uPA receptor (uPAR) [16], which allows uPA to induce pericellular plasminogen activation that enables cell migration. These uPA properties led to the erroneous conclusion that its activity was limited to the extravascular space [17], a conclusion that has persisted [18] and helped set back recognition of uPA's role in intravascular fibrinolysis.

Nevertheless, evidence has long been available that uPA has an important function in intravascular fibrinolysis since it is actually the dominant activator in the system. For example, tPA and uPA gene knockout studies in mice showed that deleting tPA alone had little effect on clot lysis whereas uPA deletion induced significant inhibition of lysis. When both tPA and uPA were deleted, a major inhibition of endogenous fibrinolysis took place [19], revealing the tPA's lytic effect requires uPA. Similarly, uPA but not tPA deletion caused spontaneous fibrin deposition in the animals, while deleting both activators induced substantial intravascular fibrin deposition [20].

The conclusion reached by both studies was that intravascular fibrinolysis requires both plasminogen activators, but that uPA had the dominant effect. This activity difference can be in part related to uPA having two fibrinolytic forms, whereas the single and two-chain forms of tPA have identical fibrinolytic activities [21]. The initiation of fibrinolysis by tPA is dependent on its fibrin binding site on lysine γ-(312-325) of the D-domain of intact fibrin [22].

As a consequence of their different properties, of tPA and uPA, they have different fibrinolytic functions which are complementary [23] and synergistic when combined both in vivo [24] and in vitro clot lysis [25]. Their activities in fibrinolysis are also sequential.

When an intravascular thrombus forms and threatens blood flow, it triggers the release of tPA from the vessel wall. The tPA binds to the thrombus at its binding site [22] and activates a fibrin-bound plasminogen at lysine Aα157 on the same domain [26]. This fibrin-tPA-plasminogen ternary complex promotes tPA's activating activity about 1,000-fold [27] enabling it to initiate fibrin degradation. Any remaining unbound tPA is rapidly cleared from plasma ($T_{1/2}$ ~5 min) and inhibited by PAI-1. This protects hemostatic fibrin, which is vulnerable to lysis by tPA

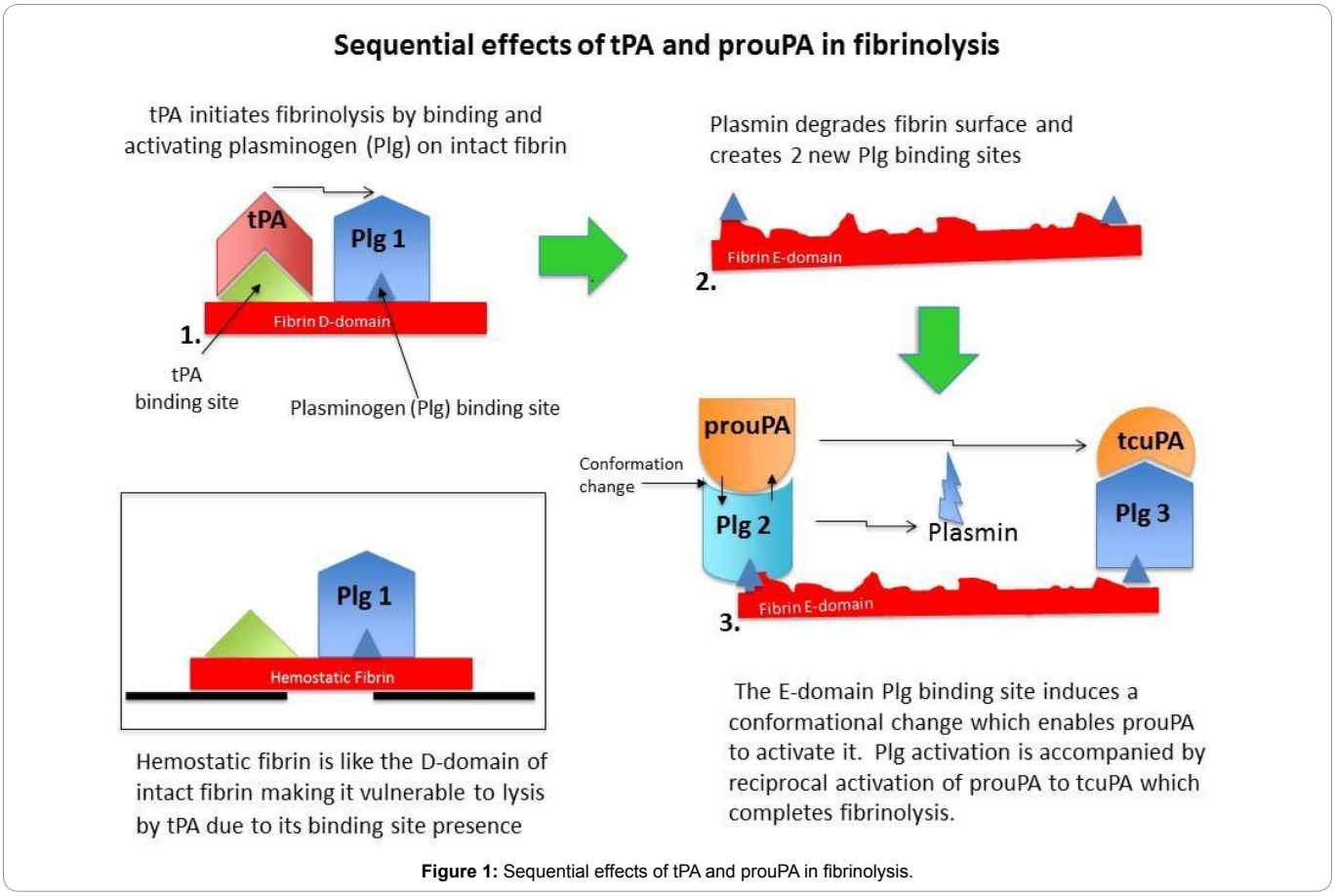

Figure 1: Sequential effects of tPA and prouPA in fibrinolysis.

since it has the same ternary complex binding sites as are on intact fibrin (Figure 1). Consequently, the principal cause of bleeding complications from tPA fibrinolysis comes from the lysis of hemostatic fibrin by high dose tPA in the circulation [28].

When fibrin degradation is initiated by tPA, it creates new plasminogen binding sites [29] that are two in number [30]. The first of these is a triple carboxy-terminal lysine binding site on the E-domain of partially degraded fibrin that induces a unique conformational change in plasminogen that binds to it. Against this conformation, the proenzyme intrinsic activity of prouPA is promoted about 250-fold enabling it to activate this plasminogen [31]. This is accompanied by reciprocal activation of prouPA by plasmin [32], and tcuPA activates the remaining fibrin-bound plasminogen completing fibrinolysis (Figure 1). Therefore, tPA activates the first fibrin-bound plasminogen and uPA the remaining two, consistent with its dominant fibrinolytic effect [19,20].

Since tPA initiates lysis on the D-domain and prouPA activates plasminogen on the E-domain, plasminogen activation in the presence of these isolated domains was tested. Plasminogen activation by tPA was promoted only by fibrin fragment-D while that by prouPA was promoted only by partially degraded fibrin fragment-E [33], consistent with the complementary modes of action of the activators [23]. This also explains why both activators are required for effective lysis at fibrin-specific doses.

The clinical bottom line of all this is that fibrinolysis requires both natural plasminogen activators working sequentially, not only tPA. When tPA is used alone, a high non-specific dose is required to in order to activate the other two fibrin-bound plasminogens that should be activated by uPA. This is less effective since the synergistic effect is absent and the risk of hemorrhagic complications is increased due lysis of hemostatic fibrin by free tPA in plasma [28].

A Clinical Proof of Concept

A test of the combination regimen was conducted in a multicenter trial of 101 patients with AMI. Since tPA initiates fibrinolysis followed by effect of prouPA/tcuPA, patients were given a mini-bolus of tPA followed by a modest prouPA infusion for 90 minutes. The first 10 patients received a 10 mg bolus of tPA, which was found to be excessive, so the remaining 91 patients received a 5 mg bolus (5% of tPA's standard therapeutic dose). Thereafter, all patients received a prouPA infusion at a rate of 40 mg/h (50 % of prouPA's monotherapy rate). Complete patency (TIMI-3) of the infarct artery at 24h was seen in 82% of the 28 patients that were re-catheterized. No reocclusions or reinfarctions were seen, reflecting the absence of a prothrombotic effect by prouPA in contrast to tPA [34]. The study mortality was 1% consistent with the high patency rate [35] and the absence of reocclusions. These results compare favorably with those reported in the best tPA trial, in which the TIMI-3 patency at 24h was 45% and the mortality was 6.3% [36].

Although these results were published in a prominent

journal, the findings gained little traction. The resistance to novel regimen, regardless of supportive evidence, is consistent with the Kuhn observation that a paradigm shift is required before any change to a well-established standard practice is considered [1]. Not long after this trial, the development of prouPA by pharma was discontinued.

Conclusions

Reperfusion of a thrombus blocked artery within two hours of the event is required for optimal salvage of heart muscle and reduction of mortality in AMI, and undoubtedly also in stroke. Such rapid reperfusion is feasible only with fibrinolysis, but tPA alone is insufficiently effective, risky, and vulnerable to reocclusions. Therefore, fibrinolysis was replaced by primary PCI as the treatment of choice in AMI and is used increasingly in ischemic stroke as well. Unfortunately, PCI almost inevitably delays reperfusion beyond the optimal salvage period. A safer, more effective fibrinolytic regimen is urgently needed and is already available in nature in our endogenous, physiological fibrinolytic system. Its therapeutic replication requires only 5% of the current therapeutic tPA dose followed by a modest prouPA infusion. A clinical proof of concept of this regimen in AMI has been completed and published.

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