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Fibrinolysis is Misunderstood which is Responsible for an Unsuccessful Therapeutic Design

Victor Gurewich*

Vascular Research Laboratory, Mount Auburn Hospital, Cambridge, Harvard Medical School, USA.
vgurewich@tsillc.net

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

Abstract

Therapeutic fibrinolysis has been synonymous with tissue plasminogen activator (tPA) for thirty years, based on the unconfirmed hypothesis that tPA alone was responsible for fibrinolysis. tPA was developed to replace streptokinase (SK), a non-specific activator, but comparative trials in acute myocardial infarction (AMI) found their benefits to be comparable except for tPA causing significantly more intracranial hemorrhage (ICH). The tPA hypothesis was contradicted by gene deletion findings in mice, which showed that fibrinolysis required both tPA and urokinase plasminogen activator (uPA)and that uPA was the dominant activator. Clot lysis studies confirmed the findings and showed tPA and uPA to have complementary effects which functioned sequentially in fibrinolysis. In combination, starting with tPA, their effects were synergistic. A sequential combination was once tested in AMI, in which 101 patients were given a mini-bolus of tPA followed by aprouPA infusion.This resulted in asix-fold lower mortality and almost two-fold higher infarct artery patency rate than that in the best of the tPA trials. Despite publication of the study in a prominent journal, the combination was never retested and fibrinolysis with tPA remained the standard. With little evidence in support of this long-standing practice, a paradigm shift is long overdue.

Introduction

An observation that is particularly relevant to fibrinolysis was made by the science philosopher Thomas Kuhn when he said that "Science does not progress as a linear accumulation of new knowledge, but undergoes periodic revolutions called paradigm shifts" [1]. In the present context this "paradigm" refers to a long-standing consensus about fibrinolysis that is so well-established that evidence inconsistent with this theory and clinical practice is resisted. As a result, the lytic treatment of vascular occlusive diseases has remained stagnant.

For example, tPA has now been used in AMI for thirty-two years and in ischemic stroke, with less success, for twenty-two years. This practice has been based entirely on the hypothesis that tPA was solely responsible for biological fibrinolysis. This concept remained untroubled by the results of tPA clinical trials which were disappointing and inconsistent with the hypothesis. An alternative fibrinolytic regimen was never tested and instead the tPA experience caused fibrinolysis itself to become discredited. As a result, fibrinolysis has been replaced by endovascular procedures, like percutaneous coronary intervention (PCI), whenever possible and PCI has become the treatment of choice in AMI.

At the same time, PCI is technically demanding, limited in its availability, and time-consuming since hospitalization is required. Coronary reperfusion is inevitably delayed by it which compromises salvage of tissue function and reduction of mortality [2]. It has been shown that in AMI, each 30 minute delay in reperfusion increases mortality by 7.5% [3]. To address this, pretreatment with tPA was extensively tested, but was abandoned when tPA was found to be incompatible with PCI in that it increased the complication rate significantly[4].

Optimally rapid reperfusion can be achieved only with fibrinolysis, but for this fibrinolysis must be made more effective, safer, and compatible with PCI.

tPA Monotherapy Differs From Biological Fibrinolysis

When tPA was first compared with SK, the non-specific activator that tPA was developed to replace, the AMI mortality with tPA and with SK were unexpectedly found to be identical [5]. A second trial gave the same results [6] and only in a third trial was a small but significantly lower mortality found with tPA in one of four groups [7]. In the three trials, a total of 95,740 AMI patients were studied to reach this small difference, and a Bayesian statistical analysis concluded that no difference between tPA and SK had been established [8]. Ironically, the only significant difference was that tPA caused more ICH than SK. Instead of the tPA hypothesis being put into question by these findings, it was concluded the results showed that "differences between different fibrinolytic regimens are unlikely to be large" [6].

By contrast to this tPA therapeutic experience, endogenous biological fibrinolysis is more effective despite a tPA plasma concentration (10-12 ng/mL) that is one thousand fold lower, and where much of it is in an inactive complex with plasminogen activator inhibitor-1 (PAI-1) [9]. Evidence of its efficacy comes from the presence of the fibrin degradation product D-dimer which is present in plasma of even healthy individuals (112-250 ng/mL).

Since D-dimer is about 60% of the fibrin monomer mass, this concentration represents a steady state of fibrinolysis of about 1 mg of fibrin being degraded. In the presence of thromboembolism, the D-dimer concentration increases as much as 30-fold indicating fibrinolysis of a corresponding amount of fibrin.

This efficacy of endogenous fibrinolysis can be explained by it being due not to tPA alone. There is now much evidence that a fibrinolytic regimen with a single plasminogen activator is inadequate because it is incomplete.

Fibrinolysis Requires both tPA and uPA

There are two plasminogen activators in blood, the second one being uPA. Since most of it is carried on the surface of platelets [10] and monocytes [11] rather than in plasma, uPA has often escaped detection. Furthermore, uPA has no fibrin affinity but instead has a cell surface uPA receptor (uPAR) by which it facilitates induction of pericellular plasminogen activation in the extravascular space[12]. This led to the belief that uPA's activity was limited to the extra vascular space [13], a belief that has persisted [14] and evidence to the contrary has generally been dismissed.

This evidence includes tPA and uPA gene knockout studies which showed that deleting tPA had no measurable effect on lysis of an intravascular clot, whereas deleting uPA did, and deleting both activators had a major effect [15]. Similarly, uPA but not tPA deletion caused spontaneous fibrin depositionin the animals and deleting both activators induced massive deposition [16]. The authors concluded that intravascular fibrinolysis required both plasminogen activators [15, 16].

Endogenous Biological Fibrinolysis Involves Both Activators

When a fibrin thrombus forms in a vessel, tPA is released at that site and binds to intact fibrin at its binding-site on γ-(312-3125) of the D-domain [17], where it activates a proximal plasminogen bound to fibrin Aα157[18]. Due to this ternary complex, tPA's plasminogen activating activity is promoted 1,000-fold [19]. Any unbound tPA is rapidly cleared from the plasma ($T_{1/2}$~5 min) or inhibited by plasminogen activator inhibitor (PAI-1). This serves the physiological function of protecting hemostat fibrin, since this fibrin contains the same tPA and plasminogen binding sites that make up the ternary complex, there by making hemostatic fibrin vulnerable to lysis. This is the mechanism principally responsible for the bleeding complications associated with tPA therapy [20].

Since no second tPA fibrin-binding site has been identified, tPA's fibrin-specific plasminogen activation is limited to this site at which it initiates fibrinolysis. Fibrin degradation then creates new plasminogen fibrin binding sites [21] that are two in number [22]. The first of these is a triple carboxyl terminal lysine binding site on the E-domain of degraded fibrin, which induces a unique conformational change in the plasminogen that binds there. Against this conformation, the intrinsic activity of the single-chain, proenzyme form of uPA, prouPA, is promoted about 250-fold enabling it to activate this plasminogen [23]. This is accompanied by reciprocal activation of prouPA to two-chain uPA (tcuPA) [24] and tcuPA activates the remaining plasminogen to complete fibrinolysis.

In this fibrinolytic design, the complementary modes of action of tPA and prouPA [25] are again evident. tPA activates plasminogen on the fibrin D-domain which is followed by prouPA activating plasminogen on the E-domain. This finding was verified by a kinetic study of plasminogen activation by these fibrin fragments. Plasminogen activation by tPA was only promoted by fragment-D, whereas that by prouPA only by fibrin fragment-E [26]. When the activators were combined, their fibrinolytic effect was synergistic [27, 28], and this effect was additionally promoted when tPA and prouPA were administered sequentially rather than simultaneously [29]. This tPA, prouPA sequence mimics that of endogenous fibrinolysis.

The fibrin-domain specificity of the activators means that fibrinolysis by either tPA or prouPA alone will require a dose high enough to induce non-fibrin-specific plasminogen activation so that the other fibrin-bound plasminogen can be activated. At these doses, bleeding side effects are significantly increased. Since the single and two-chain forms of tPA have identical activities [30] and has only a single fibrin binding site it has a single function. By contrast, uPA has s double function, having both a proenzyme and an enzyme form [31].

A Clinical Test of a Low Dose Sequential Administration of tPA and ProuPA

In a multi-center study of 101 patients with AMI fibrinolytic treatment was administered with a min-bolus of tPA followed by a prouPA infusion. The first 10 patients received a 10 mg bolus of tPA but this was found to be excessive so a 5 mg bolus (5% of the standard tPA dose) was administered in the remaining 91 patients. This was followed by an infusion of prouPA, 40 mg/h for 90 minutes (50 % of the monotherapy rate). A complete (TIMI-3) patency of the infarct artery at 24 hours was obtained in 82% of the 28 patients re-catheterized, and the overall mortality was 1% [32]. This compared favorably with the best of the tPA trials in which the TIMI-3 patency at 24h was 45% and the mortality was 6.3% [24].

Although these exceptional results were described in a prominent journal, a second study with this combination was never done, and fibrinolysis with tPA monotherapy was unaffected and remains the fibrinolytic regimen of choice.

Conclusions

Prompt reperfusion of a thrombus blocked artery is essential for optimal salvage of heart or brain function and mortality reduction. Fibrinolysis is the only way this can be achieved rapidly. For thirty years fibrinolysis has meant tPA monotherapy which is inadequately effective and risky, causing fibrinolysis to become discredited and replaced by a vascular procedure whenever possible. This inevitably delays reperfusion under mining its potential benefit. By using the biological complementary and synergistic properties of tPA and uPA in a sequential combination, fibrinolytic therapy can be made more effective and safer. This was already tested and validated in a clinical trial of AMI but its recognition and utilization awaits the paradigm shift referred to by Thomas Kuhn.

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