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Fibrinolysis with tPA Failed Because the Mechanism of Action of both was Misunderstood

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ABSTRACT

Therapeutic fibrinolysis has used tissue plasminogen activator (tPA) since 1987 based on a belief that tPA was responsible for biological fibrinolysis. This belief, however, was belied by clinical experience with tPA showing that it was not an effective fibrinolytic. Comparative clinical trials in almost 100,000 patients with acute myocardial infarction (AMI) failed to show that tPA was unequivocally more effective than streptokinase (SK), an indirect, inefficient, and non-specific plasminogen activator. Instead, it was found that tPA caused significantly more intracranial hemorrhage (ICH) side effects than SK. This disappointing experience led to the abandonment of fibrinolysis and its replacement by percutaneous coronary intervention (PCI) for AMI. However, PCI is a time-consuming, hospital procedure, poorly adapted to salvaging function of an ischemic myocardium, for which success is critically time dependent. Fibrinolysis remains the fastest method available for this and its abandonment is predicated on fibrinolysis and tPA being identical. This assumption, however, is contradicted by evidence that fibrinolysis requires both biological plasminogen activators, and that urokinase plasminogen activator (uPA) is the dominant of the two. This was also documented in a single clinical study in AMI, in which tPA's fibrinolytic function was found to be analogous to that of the starter in an automobile.

Introduction

The science philosopher, Thomas Kuhn, showed that "Science does not progress as a linear accumulation of new knowledge, but undergoes periodic revolutions called paradigm shifts." Such a paradigm shift is long overdue in therapeutic fibrinolysis, which has not changed in 33 years. tPA was given FDA approval for treatment of AMI in 1987 and has been used alone for fibrinolysis ever since, despite its disappointing efficacy and intracranial hemorrhage (ICH) side effects. Its use was based on an assumption that tPA was responsible for biological fibrinolysis.

Since fibrinolysis is an important biological defense mechanism, the finding that in its clinical use tPA has required such high doses (in clinical trials up to 150 mg was tested), puts into question the belief that tPA is the activator responsible for biological fibrinolysis, as also does its disappointing therapeutic effect. For example, even after three comparative trials in a total of 95,740 patients with AMI [1-3], it was not possible to establish that tPA was unequivocally better than SK [4]. In these studies, the tPA dose that was found to be required gave a plasma concentration about 1,000 fold higher than tPA's physiological one. Even this was not effective. However, these findings failed to bring about a paradigm shift and tPA remained the unquestioned fibrinolytic of choice.

Gene deletion studies

Gene knockout studies in mice showed that deleting the tPA gene had no measurable inhibiting effect on lysis of an intravascular clot and did not induce significant spontaneous fibrin deposition. By contrast, when the uPA gene was deleted, both inhibition of clot lysis and spontaneous fibrin deposition occurred. When

both genes were deleted, a significantly stronger effect on both of these measures took place. The investigators concluded that both activators were required for a full fibrinolytic effect [5] but that uPA rather than tPA had the dominant effect [5,6]. This finding reflects uPA having two functional states, single-chain proenzyme, and a two-chain enzymatic form, whereas tPA's single and two-chain forms have the same activities [7]. Although the gene deletion studies were published as much as 20 years ago in prominent journals, the findings were ignored.

The complementary modes of action tPA and prouPA requires both for an optimal effect

Clot lysis studies in human plasma were consistent with the gene deletion studies. For example, the double gene knockout effect is explained by tPA and prouPA's complementary modes of action giving them a synergistic fibrinolytic effect in combination [8].

This effect was tested in the PATENT study in which 101 patients with AMI given the sequential combination of activators [9]. Since tPA was found responsible for the initiation of fibrinolysis, treatment in this study was initiated by a small, 5 mg bolus of tPA (5% of the current monotherapy dose) followed by a 90-minute venous infusion of one half of the current therapeutic dose of prouPA (40mg/h). This combination induced a TIMI-3 patency at 24 h in 82% of patients and a mortality of 1%. This compares favorably with the results in the largest tPA clinical trial (GUSTO) in which the TIMI-3 patency at 24h was 45% and the mortality was 6.3% [10].

Despite this high infarct artery patency and 6-fold reduction in mortality by the tPA, prouPA combination, the study findings had no effect on fibrinolytic therapy and a second study was never done. Farmitalia, the company responsible for this trial, was sold to Pharmacia at the time, which probably had an influence on this successful trial not being followed up.

Endogenous fibrinolysis also utilizes both activators

The endogenous fibrinolytic system functions efficiently with a tPA plasma concentration of only 10-12 ng/mL, much of which is in an inactive complex with its inhibitor, plasminogen activator inhibitor-1 (PAI-1) [11]. Nevertheless, lysis is sufficient to generate a concentration of 112-250 ng/mL of D-dimer in normal individuals. Since D-dimer represents ~60% of the fibrin monomer mass, this plasma concentration represents a steady state level of ongoing fibrin degradation of ~1 mg of fibrin. In patients with thromboembolism, D-dimer levels of $\geq$ 5,000 ng/mL are found, corresponding to of $\geq$ 25 mg of fibrin being degraded. In 15% of patients with AMI, the coronary thrombus responsible for the infarct was absent by the time the patient came to catheterization for primary PCI [12], representing the rate of endogenous fibrinolysis. This spontaneous TIMI-3 patency rate compares with one only three-fold greater at 24 h by tPA at a therapeutic concentration [3].

The efficacy of endogenous fibrinolysis cannot be explained by the small, physiological amount of tPA available, most of which is in an inactive inhibitor complex with PAI-1. Instead, it consistent

with it being due to the sequential effect of tPA and prouPA in a synergistic combination, which is also consistent with findings from gene deletion [5,6] and clot lysis studies [13,14]. Since prouPA is a proenzyme, it is not inhibited by plasma inhibitors, most of which are carried by platelets [14].

The molecular function of biological fibrinolysis

The source of tPA in fibrinolysis is the vessel wall where it is stored and from where it is released at the site of an intravascular fibrin thrombus. The tPA then binds to the fibrin thrombus due to its exceptionally high affinity, a property which sets tPA apart from other plasma proteins [1]. The unbound tPA is rapidly cleared from the circulation (T1/2 ~5 min) or is inhibited by PAI-1, suggesting that free tPA is hazardous, which it is to hemostatic fibrin to which it can bind resulting in its disruption. This is the principal cause of bleeding during tPA therapy [15].

Therefore, tPA activates the first plasminogen on intact fibrin and which is close to tPA's binding site. Lysis creates two additional plasminogen binding sites, and their respective plasminogens are activated by prouPA and tcuPA.

Thus, tPA's fibrin specificity is to plasminogen on the D-domain of intact fibrin where a ternary complex is formed between fibrin, tPA, and plasminogen. By contrast, prouPA's specificity is to plasminogen on the fibrin E-domain of partially degraded fibrin a plasminogen conformational change occurs. This fibrin-domain specificity was confirmed by a kinetic study with isolated fibrin fragments D and E. Plasminogen activation by tPA was promoted only by fibrin fragment-D and that by prouPA was promoted only by the fibrin fragment-E [16], confirming that their fibrinolytic effects are complementary and sequential. Both are required for a full effect to be also fibrin-specific so that bleeding complications are minimized or eliminated.

Conclusions

Prompt reperfusion of a thrombus blocked artery is essential for optimal salvage of heart or brain function and to reduce AMI and stroke mortality. Fibrinolysis is the fastest and therefore potentially best treatment. However, the longstanding use of tPA monotherapy has been inadequate but is related to a misunderstanding of biological fibrinolysis, which requires both tPA and prouPA, rather than tPA alone. The latter is insufficiently effective and hazardous due to the large tPA doses required; twenty-fold higher than with both activators. PCI is too time-consuming for optimal salvage of function of ischemic myocardium. Therapeutic fibrinolysis modeled on the biological fibrinolytic paradigm is more effective and safer due to the lower doses required by synergy. The efficacy of this paradigm was validated in single trial in AMI in which the infarct artery patency was doubled, and mortality was reduced 6-fold [9]. A paradigm shift in therapeutic fibrinolysis is over-due.

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