# A New Approach to Thrombolysis

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## Abstract

For more than 25 years thrombolysis has been synonymous with the administration of tPA. This is reflected by the fact that in most clinical articles on fibrinolysis or thrombolysis over the last decade, the activator is no longer identified since it is assumed to be tPA. Moreover, the choice of activator has also become of little interest because it is believed that all activators have similar clinical effects.

## Introduction

This idea originated in the mega trials of the early '90's in which tPA and streptokinase (SK) were compared in patients with acute myocardial infarction (AMI). Since tPA is a fibrin-specific plasminogen activator with a direct mode of action and SK is non-specific and has an inefficient, indirect mode of plasminogen activation, it was assumed that tPA would be far more effective and clinically beneficial. However, to the surprise of the investigators, it required three mega trials with a total of 95,740 patients to show a statistically significant difference between tPA and SK. In the first two trials, the AMI mortality was the same with tPA and SK [1,2]. In the last trial, which was divided into four groups, in one of them in which an accelerated infusion rate and heparin were used, statistically significant lower 30 day mortality (6.9 vs. 7.8%) with tPA was found [3].

These results did not satisfy a Bayesian statistical analysis, which concluded that a difference between tPA and SK had not been established [4]. An additionally surprising finding in these trials was that the intracranial hemorrhage (ICH) rate with tPA was significantly greater than with SK, despite the fibrin specificity of tPA [1-3].

The investigators concluded from this that the "mortality differences…from different fibrinolytic regimens are unlikely to be large" [2]. In other words, that there was little clinical difference to be expected from different plasminogen activators or different combinations of activators. This conclusion was accepted without discussion, which, not surprisingly, had a discouraging effecton both investigators and the pharmaceutical industry and little work in thrombolysis has taken place since.

Instead, tPA and its mutant forms have remained the only thrombolytics in use. Eventually, the disappointing clinical effects and ICH risk of tPA foretold by the mega trial resulted in percutaneous coronary intervention (PCI) replacing fibrinolysis as the treatment of choice in AMI. Endovascular procedures are now also being used in ischemic stroke wherever possible. In pulmonary embolism, only patients with hemodynamic instability are considered to be candidates for thrombolysis due to its bleeding hazard.

## The Mega Trial Results were Misinterpreted

The conclusion that the mega trial results meant that little difference can be expected from different fibrinolytic regimens was based on a misunderstanding of the cause for the similar clinical effects of tPA and SK. The cause, in fact, can be attributed to the very nature of their different mechanisms of action as a result of which each activator was handicapped, but for a different reason for each. The fact that their net effects were similar was happenstance and should not have been interpreted as meaning that all fibrinolytic regimens were necessarily going to be comparable. The opposite was, in fact, potentially the case since the findings indicated that the tPA handicap needed to be understood so that it could be corrected.

SK is handicapped not only by its non-specificity but also because of its indirect mechanism of action mechanism of action. SK is not itself a plasminogen activator, but it becomes one when it forms a 1:1 complex with plasminogen (or plasmin) thereby consuming the enzyme responsible for fibrinolysis. In view of its inefficient fibrinolytic mechanism, it was especially surprising that tPA turned out to be little better than SK, indicating that it was similarly handicapped. The mechanism responsible for this is related to its high fibrin affinity, the property responsible for its fibrin-

specificity and that made it such an attractive plasminogen activator. When tPA binds to fibrin, it is to a specific binding site located adjacent to the plasminogen binding site on Lysine Aα-157 on the D-domain of intact fibrin [5]. As a result, its plasminogen activating activity is promoted about 1,000-fold [6]. However, this promotion is limited to this one fibrin site [5, 7].

In order for tPA to activate the remaining plasminogens on fibrin, which are two in number [8], very high doses of tPA are needed. This is because in the absence of this D-domain fibrin promotion, tPA is a weak plasminogen activator. At the high tPA doses required, ICH complications can occur due to tPA'slysis of hemostatic fibrin since it contains the fibrin D-domain binding site. Hemostatic fibrin is located at vessel repair sites this hazard limits the amount of tPA that can be given with reasonable safety.

Therefore, tPA monotherapy is limited by its fibrin-specific mechanism of action which caused its clinical effects to be little better than that of SK. The surprising nature of these results also provided an opportunity to identify the problem with tPA monotherapy. However, since the results were essentially dismissed at the time, the occasion was lost and thrombolysis by tPA monotherapy has continued to the present time.

The general acceptance of the idea that all plasminogen activators were comparable, not surprisingly, discouraged further scientific and pharmaceutical interest in this therapeutic modality. As a result, there was little further development, and interest in fibrinolytic the rapiesfaded. Eventually, due to continued disappointing results foretold by the mega trials, tPA was replaced by endovascular procedures as the treatment of choice in AMI, and these procedures are now also being used in ischemic stroke. Since these procedures requires hospitalization in places where expertise is available, they are too time-consuming for optimal salvage of ischemic myocardial or brain tissue, for which the greatest reduction in AMI mortality and post AMI morbidity is achieved when coronary reperfusion takes place within 1-2 hours of symptom onset [9,10]. This objective for both AMI and stroke can only be achieved by thrombolysis.

## How Thrombolysis can be Improved

The restricted mechanism of action of tPA indicates that it cannot alone be responsible for fibrinolysis, which is a remarkably efficient biological defense pathway. This is evidenced by the presence of the fibrinolytic product, D-dimer that is invariably present in blood. Its normal presence (112-250 ng/mL) indicates that fibrinolysis must be an ongoing physiological vascular repair process. Since D-dimer represents ~60% of fibrin, its normal level corresponds to a steady state of ~1 mg of fibrin lysed. In the presence of venous thromboembolism, the D-dimer level can go up to more than 5,000 ng/mL, or ~15 mg of fibrin lysed. Since most of the tPA in blood is in the form of an inactive inhibitor complex [11], it is evident that a second plasminogen activator must be involved.

The other fibrin-specific plasminogen activator is prourokinase (pro UK), which is a natural constituent of blood. However, since most proUK is bound to platelets [12,13] or monocytes [14] and only a small amount is free in plasma, it has usually been overlooked. In addition, pro UK is inactivated by thrombin so that when pro UK is experimentally included in clot formation, it can be destroyed and escape detection. Another misleading factor was the discovery of a pro UK cell receptor (UPAR) important for pericellular plasminogen activation and cell migration, a vital biological process. This finding

led to the erroneous conclusion that pro UK is only an extravascular rather than an intravascular plasminogen activator [15]. The idea has persisted and resisted change despite much evidence to the contrary [16].

For example, gene knockout studies showed that knocking out pro UK had a significantly greater effect on the suppression of lysis of a venous thrombus than did knocking out tPA which had little effect [17]. This predominance of the pro UK effect is actually consistent with the fact that both pro UK, the single-chain proenzyme, and urokinase (UK), its two-chain enzymatic form, are involved in fibrinolysis. By contrast, tPA has only one effect since its single and two-chain forms have the same enzymatic and fibrinolytic activities [18]. In fact, tPA's single function in fibrinolysis is to initiate fibrin degradation which it does when it binds to the D-domain of intact fibrin [5]. Therefore, using tPA alone for fibrinolysis can be seen as somewhat analogous to driving a car on its starting motor, pro UK and UK are essential for the completion of fibrin-specific fibrinolysis.

## The Physiological System of Fibrinolysis

When an intravascular thrombus forms, tPA in the vessel wall at that site is released, and due to a high fibrin affinity [19] binds to its binding site on the intact fibrin D-domain. It initiates fibrinolysis at that site and this creates two new plasminogen bindings sites [20]. The first of these is on the fibrin E-domain and pro UK has a high substrate affinity for plasminogen bound to this site [21]. Since pro UK has no fibrin-affinity, it is this substrate affinity which determines its fibrin-specificity, and pro UK thereby spares intact fibrin such as that in hemostatic fibrin which is vulnerable to tPA.

Activation of plasminogen at this fibrin E-domain site is accompanied by a reciprocal activation of pro UK to UK by plasmin [22]. UK then activates the remaining fibrin-bound plasminogen completing fibrinolysis. Therefore, tPA activates one plasminogen and pro UK/UK two. As a result, when fibrin-specific clot lysis rates by tPA and pro UK were compared, that by pro UK was invariably twice that by tPA [23]. This observation is consistent with the number of plasminogens found bound to intact versus degraded fibrin. There is only one on intact fibrin that is activated by tPA, whereas there were two found on degraded fibrin [8] and these are activated by pro uK/UK.

## A Clinical Test of the Physiological Fibrinolytic Paradigm

Since tPA has a high fibrin affinity and is an enzyme, it is poorly adapted to administration by a prolonged infusion, which is the way it has been administered. By contrast, it is well suited for bolus administration. Since its only function is to initiate fibrinolysis, relatively little is also needed.

Based on these considerations, 101 patients with AMI were treated either with a 10 mg bolus of tPA (10 patients) and the remainder a 5 mg bolus (5% of the monotherapy dose) PATENT trial [24]. This was followed by an infusion of pro UK, 40 mg/h for 90 minutes (50% of the monotherapy infusion rate). Complete coronary artery patency (TIMI-3) at 24h was seen in 82% of the patients, which compares with 45% in the best of the tPA trials, GUSTO [25]. In accordance with this reperfusion difference, the mortality was 1% in PATENT and 6.3% in GUSTO.

The success of the PATENT trial validated the adoption of the natural fibrinolytic paradigm for therapeutic fibrinolysis. However,

although this successful trial was published in a prominent journal, it received little notice and resulted in no reevaluation of standard fibrinolytic therapy. Monotherapy with tPA has continued, representing another example of resistance to a paradigm shift from a long-established practice.

Monotherapy trials with pro UK were also undertaken and these showed that at therapeutic concentrations in plasma, pro UK activated plasma plasminogen and plasmin in turn activated pro UK to UK, risking bleeding complications. This plasma instability of pro UK eventually resulted in Gruenenthal's application to the EMEA in Europe for a marketing license to be turned down in 1999. This was the only reason given for the decision since pro UK's clinical efficacy was comparable to that of tPA and SK and no other side effects were seen.

Therefore, there was persuasive evidence that monotherapy with either tPA or pro UK was inadequate. There was also evidence that the activators were complementary in their effects [26] and are, therefore, synergistic in combination [27]. As a result, in combination significantly lower and safer doses of each can be used, as shown by the PATENT trial [24]. However, since pro UK was the principal driver of fibrinolysis, its plasma instability, which undermined its safety and efficacy, had to be addressed.

## Mutant pro UK

The plasma instability of pro UK was related to its relatively high intrinsic activity which generated plasmin. Structure function studies determined that the intrinsic activity of pro UK was related to a flexible loop which contained a charged residue, Lys300, at its tip. A Histadine substitution at this site (Lys300→His) reduced the intrinsic activity 5-fold [28] but did not change its mechanism of action [29]. The synergistic fibrinolytic effect of pro UK with tPA [27] was also retained by the His pro UK mutant [30].

The improved plasma stability of His pro UK was due in part to its lower intrinsic activity, but was also related to its enzymatic form, His UK, being inhibited by C1-esterase inhibitor in plasma [31,32]. This is a relatively plentiful inhibitor which is also an acute phase reactant, so that it is apt to be enhanced in patients needing fibrinolytic therapy.

By substituting His pro UK for native pro UK, the physiological fibrinolytic properties of the latter can be preserved and utilized at pharmacological doses. Since the physiological paradigm calls for the sequential administration of a bolus of tPA followed by an infusion of pro UK, this is how His pro UK should be used in therapy. Moreover, since the PATENT study [24] has already shown that a 5 mg bolus is sufficient to initiate fibrin degradation, this is the tPA dose that should be used. This small dose administered as a bolus is unlikely to cause any hemorrhagic side effects, since the tPA half-life is only 5-6 minutes.

## Conclusion

Traditional thrombolysis with tPA has been plagued by inadequate efficacy and a too high risk of ICH leading to its abandonment and replacement by endovascular procedures like angioplasty whenever possible. However these hospital procedures are time consuming and too slow for salvage of ischemic myocardial tissue, which requires reperfusion within 1-2 hours of symptom onset. This can only be achieved by thrombolysis which is sufficiently simple and rapid to be administered out of hospital and in time. Thrombolysis must also be made more effective and safer, and this can be done by following

the natural synergistic fibrinolytic paradigm starting with a small (5 mg) bolus of tPA to initiate the lytic process followed by an infusion of His pro UK, for additional safety, for a night minute infusion. This sequence has been shown highly effective, as judged by the PATENT results and to be safe enough to be initiated without pre-testing, since if a bleed is encountered, the His pro UK infusion should do little harm.

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