The future of thrombolysis in the treatment of acute myocardial infarction

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The ability of thrombolytic therapy to lower mortality in patients with acute myocardial infarction was first demonstrated in 1986 by the Gruppo Italiano per lo Studio della Streptochinasi nell'Infarto Miocardico. In the ensuing 10 years, large efforts have been undertaken to develop more effective and safer thrombolytic agents. In addition, the value of adjunctive agents influencing thrombotic and thrombolytic processes was demonstrated, and newer agents are under active investigation. This review focuses on theoretical and practical aspects of optimizing thrombolytic therapy and on genetically engineered third generation plasminogen activators. Optimized thrombolytic therapy may make this form of therapy available to patients who are currently considered ineligible, and it will lead to earlier, more complete reperfusion of infarct-related coronary arteries. The benefits and risks of optimized thrombolytic regimens relative to those of mechanical reperfusion strategies will require constant reassessment while both forms of treatment develop.

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Introduction

The pathophysiology of acute myocardial infarction (AMI) was clearly identified in 1980 as thrombotic occlusion of a coronary artery. Dissolution of a coronary thrombus depends on the action of an endogenous serine protease, plasmin, which is generated from its precursor zymogen, plasminogen, by enzymes known as plasminogen activators. The basis of thrombolytic therapy is the intravenous administration of an exogenous plasminogen activator, which causes the generation of plasmin, lysis of the occluding thrombus, and restoration of flow to the area of ischemia. Because of the limited ischemic survival time of myocardial tissue, lysis must occur rapidly to prevent necrosis, limit infarct size, and reduce mortality. In the past, this has necessitated the administration of large doses of plasminogen activators, well beyond the threshold of fibrin specificity even of relatively fibrin-specific thrombolytic agents. Under normal circumstances, plasmin generated in the circulation is rapidly inactivated by α2-antiplasmin; however, when the fibrinolytic system is extensively activated, α2-antiplasmin and second line inactivators can be consumed completely. Excess free plasmin then degrades clotting factors in an uncontrolled fashion, which leads to a severe haemostatic defect.

Clinical efficacy and limitations of currently used thrombolytic regimens have come to light in several large trials. Administration of streptokinase, recombinant tissue type plasminogen activator (alteplase, t-PA), or anisoylated plasminogen streptokinase activator complex (APSAC, anistreplase) lowers mortality. The Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries trial (GUSTO) showed that t-PA was superior to streptokinase in lowering mortality. Furthermore, it demonstrated that both the rapidity by which patency is obtained was superior to streptokinase in lowering mortality. In addition, it demonstrated that both the completeness of patency obtained, as well as the completeness of patency obtained, correlate with mortality. The more rapid and complete the restoration of flow, the better the clinical outcome will be.

The best currently available regimens for thrombolytic therapy are associated with limitations: (1) Overall patency (perfusion grades 2 and 3 from the Thrombolysis in Myocardial Infarction trials [TIMI]) is achieved in only 85% of infarct-related arteries. (2) Complete patency with normalized flow (TIMI grade 3 flow) is achieved in only 60% of infarct-related arteries. (3) TIMI grade 2-3 flow is not obtained until 90 min after initiation of therapy; the patency rate before 90 min is considerably lower. (4) Complications include bleeding — most notably intracranial bleeding, which...
occurs in 0.5–1.5% of patients. (5) These results are obtained in selected patients because 30–60% of all patients with AMI are excluded from thrombolytic therapy.

**Approaches to improvement of thrombolytic therapy**

Several lines of investigation are currently being pursued to enhance the efficacy and specificity of thrombolytic therapy.

The interval between onset of symptoms and start of treatment has been reduced through general organizational approaches, such as patient education (e.g. a media campaign to present early when symptoms of myocardial ischaemia occur) and 'fast-track' diagnosis of patients with chest pain in the clinic\(^{19}\). Thrombolysis in a pre-hospital setting is also likely to reduce the duration of ischaemia, at least in rural regions where transport time is lengthy\(^{10,11}\). The possibility of administering thrombolysis before hospital admission is an advantage of this therapy over direct percutaneous transluminal coronary angioplasty (PTCA) that must be taken into account in future comparative trials. Time gained by improved management of AMI is just as precious as time gained by superior thrombolytic agents.

Contraindications to thrombolytic therapy have been revised several times. Thrombolysis has been shown to be effective in most patients \(\leq 12\) h after the onset of pain, and patients should not be excluded from thrombolysis on the basis of age alone\(^{12}\). Also, no data preclude treatment of patients with diabetes, even those with diabetic retinopathy\(^{13}\). Thus, as our knowledge about the true risks of presumed contraindications expands, some current contraindications are likely to be revoked, which will result in a larger population eligible for thrombolytic therapy.

The value of adjunctive therapy was clearly demonstrated in the Second International Study of Infract Survival (ISIS-2) study. Aspirin administration was associated with a reduction in mortality additive to that achievable with streptokinase\(^{14}\). It is likely that more potent inhibitors of platelet action such as platelet fibrinogen receptor (GP IIb/IIIa) antagonists\(^{15}\) will increase the speed and degree of lysis and lessen re-thrombosis after cessation of thrombolytic therapy.

More potent inhibitors of thrombin that can inhibit fibrin-bound thrombin (which is inaccessible for the heparin antithrombin III complex) are also likely to have an impact on the future use of thrombolysis. Dosing appears to be a critical issue, as suggested by an unacceptably high rate of intracranial haemorrhage in three studies with hirudin\(^{16}\).

The tools of molecular biology and genetic engineering have been used to modify plasminogen activator function, and the advances that result from these approaches are the focus of this review. Most interest has been devoted to consideration of plasma half-life, fibrin specificity, and antigenicity. Although the naturally occurring plasminogen activators, t-PA and single chain urokinase plasminogen activator (scu-PA), have a short half-life in patients (4–6 min), the results of the majority of studies in experimental animals and most recent clinical studies suggest that efficacy is probably improved, without impairment of safety, when half-life is prolonged\(^{17,18}\). On a practical level, a plasminogen activator with a longer half-life could be administered as a bolus, which facilitates administration, rather than as an infusion. Bolus administration may be more of an advantage for thrombolytic therapy in a pre-hospital setting than in the intensive care unit.

Whether fibrin specificity is a desirable feature of plasminogen activators has been much debated. Theoretical advantages of fibrin-specific agents include faster action, fewer systemic side effects, and less bleeding. On the other hand, non-specific plasminogen activation in theory could increase lysis and prevent re-occlusion by generation of fibrin(ogen) degradation products and cause less bleeding and stroke through decreased lysis of fibrin plugs. At issue is the observation that patients with AMI who are treated with t-PA are at a slightly increased risk of stroke compared with patients treated with streptokinase\(^{60}\). The mechanism responsible for this difference is not completely understood at present.

Although antigenicity is an obvious problem of streptokinase, it is also a potential limitation for any genetically modified plasminogen activator. The potential for evoking an immune response is difficult or impossible to predict before clinical evaluation.

As a result of the application of recombinant methods to tailor plasminogen activator function, hundreds of modified enzymes have been developed and evaluated. The promise of these potential therapies is well illustrated by selected agents that represent the spectrum of clinical and basic research activity in this area.

**Reteplase**

Reteplase (r-PA) is characterized by an increased half-life (18 min vs 4–6 min) and reduced fibrin affinity in comparison with native t-PA. These properties were obtained by deletion of the first kringle domain and the finger and growth factor domains of the native molecule\(^{19}\). In clinical studies conducted to evaluate the efficacy of t-PA in the treatment of patients with AMI, a regimen consisting of two intravenous boluses of 10 megaunits (MU) of r-PA injected 30 min apart was superior to conventional t-PA administration (100 mg infused over 180 min) with regard to both speed and completeness of thrombolysis. TIMI 3 patency at 90 min was 62.8% for r-PA compared to 47.6% for t-PA \((P=0.01)\)^{17}. The same regimen of r-PA also showed results superior to those with the accelerated administration of t-PA (GUSTO regimen, 100 mg infused over 90 min); TIMI 3 patency at 90 min was 59.9% for r-PA.
and 45.2% for t-PA (P=0.05), and patients were apparently not at higher risk for side effects. In a large mortality study, t-PA proved to be at least equivalent to streptokinase in reduction of mortality.

**Staphylokinase**

Recombinant staphylokinase consists of 136 amino acids in a single polypeptide chain. The molecular weight is approximately 16.5 kD. Like streptokinase, staphylokinase is not an enzyme and does not convert plasminogen to plasmin directly. It forms a 1:1 stoichiometric complex with plasminogen, and this inactive complex is converted to the active plasmin staphylokinase complex. In contrast to the plasminogen streptokinase complex, the plasminogen staphylokinase complex is rapidly neutralized by α2-antiplasmin in plasma in the absence of fibrin; thus excessive systemic plasminogen activation is avoided. In the presence of fibrin, the complex is relatively resistant to α2-antiplasmin neutralization and thereby provides for some fibrin specificity. In vitro experiments demonstrated that staphylokinase is a potent plasminogen activator that compares favourably with streptokinase in terms of fibrinolytic efficacy, fibrin selectivity, and potency toward platelet-rich clots. In animal experiments, these properties could be confirmed; however, the immunogenicity of staphylokinase may limit its application.

In a pilot trial, 10 patients with AMI received 10 mg of staphylokinase infused over 30 min in combination with heparin and aspirin. Recanalization was achieved in nine of 10 patients (TIMI 3 in eight patients), and patency was maintained in all but one patient at 24 h. No measurable change occurred in concentrations of plasma fibrinogen and α2-antiplasmin. A larger pilot trial in 100 patients with MI has been completed, and the agent is presently also being evaluated in thromboembolic peripheral arterial occlusion.

**t-PA–scu-PA chimera**

A large number of recombinant chimeric plasminogen activators have been constructed by using different domains of t-PA and the serine proteinase domain of scu-PA. A chimera consisting of the kringle 1 and kringle 2 domains of t-PA (amino acids 87–274) fused with the serine proteinase domain of scu-PA (amino acids 144–411) demonstrated markedly increased thrombolytic potency when compared with both parent molecules in different animal models. However, these findings were mainly due to a prolonged half-life. Results of a clinical feasibility study in six patients with AMI suggest that administration of two 10 mg boluses of this agent can induce rapid, specific thrombolysis.

**bat–t-PA**

The saliva of the vampire bat *Desmodus rotundus* contains a family of potent plasminogen activators named *Desmodus* salivary plasminogen activators (DSPAs) after their original source. For its unique thrombolytic properties, DSPA α1 was chosen for further development. DSPA α1 shows high structural homology with t-PA but it has only one kringle structure that resembles kringle 1 of t-PA. It lacks the plasmin cleavage site present in t-PA and scu-PA, making it the only known plasminogen activator that exists exclusively as a single chain molecule possessing full catalytic activity. DSPA α1 was shown to be a potent and fibrin-specific thrombolytic agent in rats with pulmonary embolism and in animal models of arterial thrombosis. No clinical data on the efficacy and safety of DSPA α1 have been reported.

**TNK mutant**

A second re-engineered molecule currently undergoing clinical evaluation is TNK. Like r-PA, TNK was constructed by altering native t-PA; however, highly specific modifications of t-PA were made rather than deletion of whole domains of the t-PA molecule. Site-directed mutagenesis via substitution of a single amino acid (T103N) was used to construct a t-PA variant that contains an additional glycosylation site in the first kringle domain. A second single amino acid substitution (N117Q) removed an existing glycosylation site on kringle 1. Finally, substitution of four amino acids in the catalytic domain (KHRR-296-299-AAAA) further increased fibrin specificity and made the molecule resistant to its naturally occurring inhibitor, plasminogen activator inhibitor 1 (PAI-1). Together, these changes result in a prolonged half-life (approximately 30 min) and 80-fold enhancement of resistance to PAI-1 as well as a 14-fold augmentation of relative fibrin specificity. In vivo, TNK proved to be 8- and 13-fold more potent than native t-PA in lysing of whole blood clots and platelet-enriched clots, respectively. TNK conserves fibrinogen and, because of its slower clearance, is effective when given as a bolus. Although TNK is currently being evaluated in clinical trials, no information about its clinical efficacy is available. Judging by its in vitro profile and by results obtained in experiments with animals, TNK is a promising thrombolytic agent.

**Antibody-targeted plasminogen activators**

Antibody targeting for the treatment or prevention of thrombi entails the engineering of a bifunctional molecule that contains both a highly specific antibody combining site for concentrating the molecule at the desired target (the thrombus) and an effector site for initiating thrombolysis or preventing additional formation of thrombus. The principle allows for variation in a number of ways, since antibodies of exquisite specificity
(e.g. recognition of only the activated platelet IIb/IIIa receptor) can be combined with a variety of plasminogen activators or antiplatelet and antithrombin agents.

With the aim to select the most potent combination of antibody and effector agents for recombinant production, model molecules were created by chemically cross-linking urokinase, t-PA, scu-PA, and hirudin to antifibrin and antiplatelet antibodies or their Fab fragments[32−36]. The most effective plasminogen activator conjugate, the combination of a fibrin specific monoclonal antibody Fab' fragment with scu-PA, demonstrated a 20-fold enhancement of thrombolytic potency in vivo over the parent molecule scu-PA[37]. This approach offers the theoretical advantage of adding fibrin binding to scu-PA, a native plasminogen activator that is fibrin selective but does not bind to fibrin and is resistant to inhibition by PAI-1. For the recombinant antifibrin scu-PA molecule, the Fab part of the antibody molecule (which contains antibody heavy chain residues 1−351) was fused in contiguous peptide sequence to low molecular weight scu-PA (residues 144−411 of scu-PA). This molecule showed a prolonged half-life, enzymatic properties very similar to those of native scu-PA (in particular the increase in activity after cleavage by plasmin to the two chain form), and fibrin binding in a manner similar to that of the native antifibrin antibody. In vitro, in a human plasma clot lysis assay, the recombinant antifibrin scu-PA molecule was six times more potent than native scu-PA. At the same time, it was more fibrin specific, as evidenced by a decreased consumption of α2-antiplasmin and fibrinogen. In vivo, antifibrin scu-PA displayed a remarkable 20-fold increase in thrombolytic potency over the entire dose-response range[38]. These results confirmed the promise of the molecule and also the value of the chemical model conjugates by which these results had been precisely predicted.

In recently completed studies in a baboon model that allows comparisons of thrombolytic potency and inhibition of thrombus deposition in relation to both the dose and the plasma concentration of each plasminogen activator, antifibrin scu-PA was 8−10-fold more potent than t-PA and 15−20-fold more potent than scu-PA (by dose administered). It is of interest that, at equipotent dosages, template bleeding times in the baboon for antifibrin scu-PA were unchanged, whereas those for scu-PA or t-PA were significantly prolonged (Runge et al., unpublished observations). Since bleeding time prolongation has been suggested as a marker for the clinical risk of haemorrhage, antifibrin scu-PA may be a safer agent as well[39]. A different molecule consisting of antiplatelet antibody 7E3 (directed against the GP IIb/IIIa receptor) and urokinase demonstrated markedly enhanced lysis of platelet-rich thrombi and potentiation of the anti-aggregatory effect of the antibody[36]. Similar studies were performed by another group of researchers with an antibody of different specificity, and similar results were obtained[40,41].

Antibody targeting of fresh vs old thrombi

The design of two additional molecules was based on the observation that a high concentration of thrombin exists in the micro-environment of a recently formed intravascular thrombus. Yang et al.[42] described an antifibrin urokinase fusion molecule in which the urokinase-derived catalytic domain was modified by site-directed mutagenesis so that the peptide bond normally cleaved by plasmin (to activate urokinase) would now be cleaved by thrombin. Thus, a selective thrombin cleavage site in a molecule targeted to fibrin would initiate clot lysis after activation by thrombin. According to its design, antifibrin scu-PA-T converted plasminogen to plasmin after activation by thrombin. In vitro clot lysis experiments showed that the inhibition of thrombin by hirudin resulted in inhibition of clot lysis by antifibrin scu-PA-T. These results suggest that antifibrin scu-PA-T has the potential of lysing fresh clots, which are thrombin-rich, in a relatively selective fashion. Older clots, which contain less thrombin, would be relatively resistant. The reported strategy may be an important step in the direction of discriminating between fresh pathological thrombi in the coronary arteries and older haemostatic plugs in gastric or cerebral arteries.

Antibody targeting of adjunctive agents

The exquisitely specific antibody-targeted plasminogen activators may need equally specific adjunctive agents to exploit their potential fully. Most recently, the synthesis of a bifunctional molecule consisting of an antifibrin antibody Fab fragment and the direct thrombin inhibitor hirudin was reported[15]. The intent was to inhibit further fibrin deposition at the sites of thrombosis or thrombolysis while systemic anticoagulation was avoided. The antibody chosen binds to the Bβ 15−22 epitope of the fibrin β-chain. This is the new amino terminus that becomes exposed only after thrombin has cleaved off fibrinopeptide B. Thus, the conjugate can specifically bind only at sites of thrombin action. In vitro observations demonstrated that compared with uncoupled hirudin, the presence of antifibrin hirudin conjugate was associated with an approximately 10-fold reduction in fibrin deposition on an experimental clot suspended in human plasma. These results have been confirmed in a non-human primate model of thrombosis (Bode et al., unpublished observations).

A similar recombinant molecule was designed with a factor Xa cleavage site between the antibody and hirudin segments of the protein. The cleavage site became necessary because hirudin only becomes fully active when both the aminoterminal and carboxyterminus are free to interact with thrombin. A factor Xa cleavage site was chosen because factor Xa cleaves at the carboxyterminal end of a four amino acid recognition
sequence (which releases a free hirudin aminoterminus) and also because it may confer additional specificity to the molecule (with higher factor Xa concentrations in the proximity of ongoing thrombosis). This molecule is currently undergoing testing.

Conclusions

The new, genetically engineered ‘third generation’ thrombolytic agents described here offer not only the promise of improved clinical outcomes but also the opportunity to determine the relative importance of fibrin specificity, plasma half-life, and resistance to inhibition by plasma inhibitors in thrombolytic therapy. In addition to increased efficacy, early studies suggested that safety may also be enhanced especially when highly selective, antibody targeted plasminogen activators and antithrombin agents are used.

However, problems in proving the superiority of newly developed approaches will remain. In general, thrombolytic therapy results in a reduction of absolute mortality in the range of 2-4% in patients with AMI. Demonstration of this benefit by comparing thrombolytic therapy with placebo required large megatrials. It is unlikely that a similar incremental benefit will be observed when new approaches are compared with current therapy, which will necessitate even larger sample sizes to show clinical benefit. Even more challenging is the task of showing a reduction in the incidence of a rare side effect, e.g. intracranial haemorrhage.

Thrombolytic therapy is the treatment that most patients with AMI will receive in the foreseeable future. Therefore, every effort should be made to optimize thrombolytic efficacy and safety. It will also be interesting to see how alternative treatment strategies, e.g. mechanical recanalization, will compare with optimized thrombolytic therapy administered in a pre-hospital setting.

References


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