

Newer Thrombolytic Agents

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Abstract

Several lines of research towards improvement of thrombolytic agents are being explored, including the construction of mutants of plasminogen activators, chimeric plasminogen activators, conjugates of plasminogen activators with monoclonal antibodies, and plasminogen activators from animal or bacterial origin. Some of these new thrombolytic agents have shown promise in animal models of venous or arterial thrombosis; only those which are being investigated in clinical studies are briefly discussed.

Monteplase is a modified tissue type-plasminogen activator (t-PA) constructed by substituting only one amino acid in the epidermal growth factor domain (Cys⁸⁴ → Ser) and expressed in baby Syrian hamster kidney cells. It has a prolonged half-life of more than 20 min, as compared to 4 min for native t-PA. TNK-t-PA differs from t-PA by 3 mutations. This mutant has increased thrombolytic potency, slower clearance and enhanced resistance to the inhibitor PAI-1. Reteplase is a non-glycosylated deletion mutant of wild-type human t-PA which contains only kringle 2 and the protease domain but lacks its kringle 1 and the finger and growth factor domains. The structural changes in reteplase translate into a decreased fibrin binding, a lower affinity to endothelial and liver cells resulting in an extended half-life. Lanoteplase is a deletion mutant of t-PA with a half-life that is circa 10 times greater than alteplase, making it suitable for single bolus injection. YM866 is another mutant of t-PA in which the amino acids 92 to 173 of kringle 1 were deleted and arginine²⁷⁵ replaced by glutamic acid which confers a longer half-life to the mutant. Recombinant glycosylated pro-urokinase has a greater stability than recombinant unglycosylated pro-urokinase, is rapid acting and safe in the clinical doses used. Staphylokinase (SAK) is produced by *Staphylococcus aureus*. It induces efficient and rapid recanalization, also after bolus injection, but is immunogenic.

There are only a few large scale clinical trials published directly comparing fibrin-selective thrombolytic drugs. In patients with acute myocardial infarction, reteplase, administered in bolus injections, is associated with a similar mortality and bleeding rate as front loaded t-PA. Bolus TNK-t-PA has a similar incidence of cerebral bleeding as front loaded t-PA and is associated with the same survival rate after acute myocardial infarction in a large mortality trial.

Ann Acad Med Singapore 1999; 28:424-33

Key words: Monteplase, Pro-urokinase, Reteplase, Saruplase, Staphylokinase, TNK-t-PA, YM866

Introduction: Why the Quest for Better Thrombolytic Agents Continues

First-generation fibrinolytic agents (streptokinase and urokinase) are effective thrombolytic drugs, but are not fibrin specific as they also have the property to convert circulating plasminogen to plasmin when given at therapeutic doses. Because the plasminogen in the thrombus is in equilibrium with the plasminogen pool in plasma, the plasminogen present into the interstices and on the surface of the thrombus is also gradually depleted. This "plasminogen steal", prominent with streptokinase (SK) and urokinase (UK), attenuates clot lysis, thereby reducing the therapeutic efficacy of these agents. Furthermore, streptokinase is immunogenic; resulting in drug resistance and can induce a temperature rise and allergic reactions.

The equimolar complex of streptokinase and acylated

human plasminogen was synthesized to improve the perceived disadvantages of streptokinase (Fig. 1). This molecule termed APSAC (Acylated Plasminogen Activator Complex) or anistreplase, can be administered as a bolus due to its longer plasma half-life compared to streptokinase. The hope for a higher fibrin (thrombus) affinity and fibrinolytic efficacy compared to streptokinase was not fulfilled in clinical trials. Moreover, the compound still elicits antigenetic responses in patients; pyrexia and hypotension are non-frequent adverse reactions.

By developing highly fibrin-specific thrombolytic compounds, the hope of the second generation thrombolytic drugs (tissue-plasminogen activator, t-PA; single-chain urokinase-type plasminogen activator, scu-PA or pro-urokinase) was to avoid the systemic lytic state resulting in fibrinogen and plasminogen depletion in circulating

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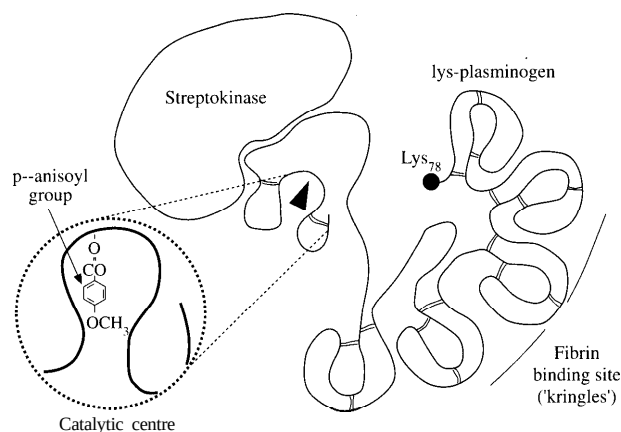


Fig. 1. Schematic presentation of the structure of APSAC. The compound is an equimolar complex of para-anisoylated human lys-plasminogen with streptokinase. The serine residue at the active centre of plasminogen is temporarily masked by a p-anisoyl group.

plasma. The underlying hypothesis was that fibrinogen depletion was the main cause of bleeding in patients treated with streptokinase, urokinase and anistreplase. Although t-PA and scu-PA administration in animal models was followed by substantially less loss of fibrinogen and plasminogen than after streptokinase administration, the doses of t-PA and scu-PA required in patients for rapid coronary thrombolysis produce a mild to moderate decrease in circulating fibrinogen. Moreover, the risk of cerebral bleeding with t-PA is slightly higher than with streptokinase (0.7% versus 0.55%). Only small numbers of patients have been treated with glycosylated or unglycosylated scu-PA, a meta-analysis of 5 studies shows an incidence of cerebral bleeding of 0.46% (11/2397).

The quest continues for better thrombolytic agents as there are still several important limitations with second generation thrombolytic drugs. A brisk coronary flow, Thrombolysis in Myocardial Infarction (TIMI) grade 3 within 90 minutes, is obtained in only half of the myocardial infarction patients treated with t-PA. To obtain this brisk anterograde coronary flow requires an average of 45 minutes and reocclusion occurs in roughly 10% of patients. In most trials the residual mortality in patients treated with thrombolytic drugs is not less than half of that without thrombolytic treatment.

In the treatment of myocardial infarction and ischaemic stroke, the delay between clinical symptoms and initiation of thrombolytic treatment is critical. Earlier and accelerated administration of thrombolytic drugs should be the first and immediately applicable goal. This does require intensified education of the population at risk and their family, of the paramedical and medical personnel but also a more efficient triage system. It is important to realize that rapid recanalization of an occluded artery may reflect not only the ease with which the thrombolytic agent reaches and diffuses into the

thrombus, and the velocity of local plasminogen activation but also the speed with which thrombolytic therapy can be initiated in the emergency room, ambulance or even at home.

These ideas have encouraged the development of "front-loaded" or "bolus" administration schedules of thrombolytic drugs. In the early years of thrombolytic therapy, a rapid countering of the thrombolytic effect was thought to be an advantage, in that thrombolysis could rapidly be interrupted if complications occurred. This has proved more difficult to achieve than anticipated, because plasminogen activation may continue within a thrombus (biological activity) after systemic thrombolytic activity in circulating plasma (half-life) has returned to normal. Conversely, agents with rapid termination of action may be unsatisfactory because it may be difficult to ensure completion of clot lysis without the technical difficulties of a carefully controlled infusion.

Another shortcoming is the lack of impact of heparin on the speed of drug-induced thrombolysis. In addition, heparin does not consistently prevent reocclusion. There is a need to use more specific and potent antithrombotic agents.

Paradoxically, the fibrinolytic drugs themselves can exert procoagulant effects through elaboration of plasmin in the fluid phase of blood that in turn activates the coagulation system directly (with conversion of prothrombin to thrombin, activation of factors X, and V). The acceleration of coagulation may result in a retardation of apparent thrombolysis, failure of initial recanalization, virtually instantaneous reocclusion masquerading as failed thrombolysis. Early thrombotic reocclusion of infarct-related arteries is known to be associated with increased mortality and a high incidence of recurrent infarction. Thus, vigorous, concomitant anticoagulation is needed. Specific reduction of platelet aggregation is presently being explored with monoclonal antibodies or synthetic peptides against the platelet glycoprotein receptors IIb/IIIa. Another approach is the use of selective direct inhibitors of thrombin (hirudin, hirulog or bivalirudin, synthetic thrombin inhibitors), specific inhibitors of factor Xa (anticoagulants derived from tick or leech or synthetic antifactor Xa molecules) or inhibitors of factor VIIa (recombinant tissue factor pathway inhibitor).

New Thrombolytic Agents in Clinical Development

Several lines of research towards improvement of thrombolytic agents are being explored, including the construction of mutants of single chain urokinase-type plasminogen activator or of tissue-type plasminogen activator, chimeric plasminogen activators, conjugates of plasminogen activators with monoclonal antibodies, and plasminogen activators from animal or bacterial

origin. Some of these new thrombolytic agents have shown promise in animal models of venous or arterial thrombosis; only those who are being investigated in clinical studies are discussed in this overview (Table I).

Mu tan ts of Tissue- Type Plasminogen Activator

Monteplase (E6010, Cleactor™ Eisai Co) is a modified t-PA constructed by substituting only one amino acid in the epidermal growth factor domain (Cys⁸⁴→Ser) and was expressed in baby Syrian hamster kidney cells.⁷ It has a prolonged half-life of 23 min as compared to 4 min for native t-PA alteplase² (Fig. 2).

Because of its longer half-life, monteplase can be injected successfully as a single intravenous bolus. The compound has been studied in animal thrombosis models and a dose-finding study was conducted in patients with acute myocardial infarction.* A dose of 0.22 mg monteplase/kg results in significantly higher rates of TIM1 grade 2 or 3 perfusion in patients with myocardial infarction than a lower dose (0.12 mg/kg). At 60 min after administration, monteplase 0.22 mg/kg was asso-

ciated with recanalization rates of 78% (TIM1 grade 2) and 69% (TIM1 grade 3). Furthermore, this dosage was associated with an acceptable incidence of bleeding events (3/58 bleeding events occurred in the 0.22 mg/kg group compared with 1/64 and 9/68 events in the 0.12 and 0.32 mg/kg groups, respectively).

In a double-blind, prospective, randomized study conducted in Japan, monteplase was shown to produce higher rates of recanalization than native t-PA (tissokinase), and to achieve recanalization at an earlier stage.³ Tissokinase has a specific activity of 500,000 IU/mg versus 600,000 for alteplase. This study involved 199 patients with myocardial infarction who were randomized within 6 hours of onset to treatment with a single intravenous bolus injection of monteplase 0.22 mg/kg over 2 min or intravenous administration of 14.4 MU1 tissokinase (28.8 mg), 10% of the total dose over 2 min, the remainder infused over 60 min. Patients received heparin 5000 IU, and coronary angiography was performed to confirm that the infarct-related artery was fully occluded. Thrombolytic therapy was then initiated and assessments of recanalization were made at 15, 30, 45 and 60 min. If the extent of recanalization was insufficient at 60 min, further intervention was performed (e.g. angioplasty).

The primary endpoint was the recanalization rate TIM1 grade 2 or 3 of the infarct-related coronary artery at 60 min after the start of treatment. Time to reperfusion was shorter in the monteplase group than in the tissokinase group. TIM1 flow grade 2 or 3 recanalization at 15, 30, 45 and 60 min after trial drug administration was observed in 37%, 62%, 74% and 79% [95% confidence interval (CI) 70% to 87%] of the monteplase-treated patients and in 14%, 32%, 50% and 65% (95% CI 55% to 74%) of native t-PA-treated patients, respectively (P = 0.032 at 60 min). This study indicated that, compared with one quarter of the recommended dose of native t-PA, a single bolus injection of monteplase over 2 min produces a higher rate of early recanalization of the infarct-related coro-

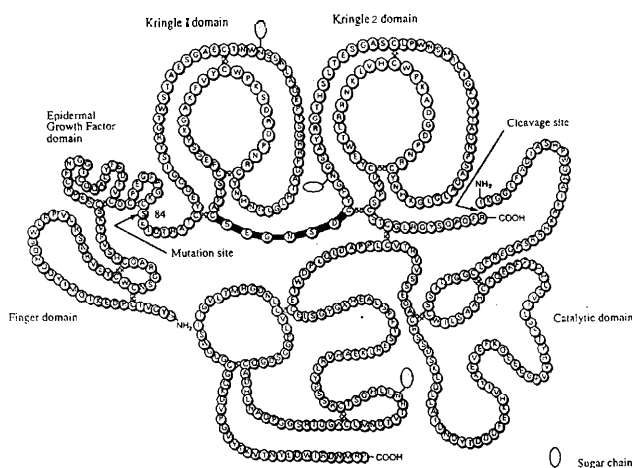


Fig. 2. Schematic representation of the primary structure of monteplase, a mutant of t-PA in which one amino acid in the epidermal growth factor domain was substituted. (Cys⁸⁴→Ser)

TABLE I: RECOMBINANT THROMBOLYTIC AGENTS IN CLINICAL USE OR DEVELOPMENT

	Alteplase	Monteplase	TNK-t-PA	Reteplase	Lanoteplase	Saruplase**	Staphylokinase
Molecular weight (D)	70,000	68,000	70,000	39,000	53,500	46,500	16,500
Plasma half-life (min)	4-8	23	20	15	23	9	6
Fibrin-specificity	++	+++	+++	+	+	±	++++
Plasminogen activation	Direct	Direct	Direct	Direct	Direct	Direct	Indirect
Dose*	100 mg/90 min	0.22 mg/kg bolus	0.5 mg/kg bolus	2 x 10 IU boluses 30 min apart	120 IU/kg bolus	80 mg/60 min	20-30 mg/30 min
Antigenicity					-	-	+
Hypotension					-	-	-
Patency at 90 min	+++	+++	+++(+?)	++++	++++	+++	+++(+?)
Haemorrhagic stroke	++	+	+or++	++	?	++	?

* Most frequently used/tested

** Recombinant glycosylated scu-PA (pro-urokinase)

nary artery without fatal bleeding complications. This is probably one of the last coronary recanalization studies (coronary angiography before and after thrombolysis) to be conducted in patients.

TNK-t-PA (Tenecteplase, Genentech) is a recombinant t-PA mutant in which Thr¹⁰³ is substituted by Asn and the sequence Lys²⁹⁶-His-Arg-Arg is mutagenized to Ala-Ala-Ala-Ala which was found to have both a prolonged half-life and resistance to plasminogen activator inhibitor type 1 (PAI-1). This mutant has an increased lytic potency on platelet-rich arterial thrombi (also rich in PAI-1) in a canine model of coronary thrombosis.⁴ Additional substitution in this mutant of Asn¹¹⁷ by Gln deletes the glycosylation site in kringle 1 whereas substitution of Thr¹⁰³ by Asn reintroduces a glycosylation site in kringle 1 but at a different locus. These modifications result in an 8-fold slower clearance and 200-fold greater resistance to PAI-1. These three combinations in a single molecule are referred to as TNK-t-PA (Fig. 3). In *in vivo* models of thrombolysis in rabbits, TNK-t-PA was shown to have an increased thrombolytic potency on platelet-rich clots, to conserve fibrinogen and to be effective upon bolus administration at half the dose of t-PA.⁵ Similar results were obtained in a combined arterial and venous thrombosis model in the dog.⁶

Although there is vast excess of t-PA over PAI-I in the circulation during thrombolytic therapy, one should remember that critical lysis occurs at the surface of an

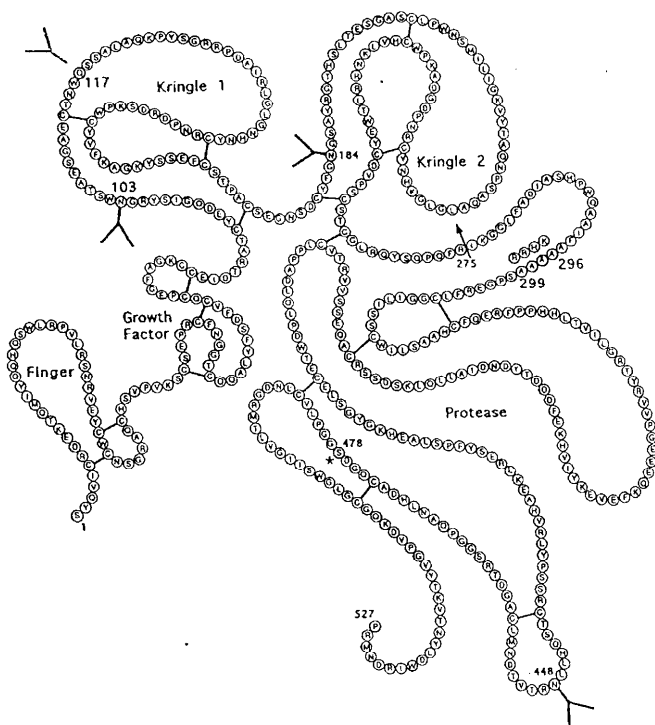


Fig. 3. Schematic representation of the primary structure of TNK-t-PA (substitution in rt-PA of Thr¹⁰³ by Asn, Asn¹¹⁷ by Gln and Lys²⁹⁶-His-Arg-Arg by Ala-Ala-Ala-Ala). The aminoacids are represented by their single-letter symbols, black bars indicate disulfide bonds, and asterisks indicate the active site residues in the protease part. The arrow indicates the plasmin cleavage site.

arterial thrombus where the PAI-1 concentration can be very high.⁷ Mutants with prolonged half-life and resistance to PAI-1 may therefore be useful to reduce the potential contribution of high PAI-1 levels to reocclusion. In patients with acute myocardial infarction, TNK-t-PA has a plasma clearance of 151 ± 55 ml/min and a half-life of 17 ± 7 min, as compared to 572 ± 132 ml/min and 3.5 ± 1.4 min for wild-type t-PA.⁸

In the TIM1 10A trial, a phase 1 dose-ranging pilot study in 113 patients with acute myocardial infarction, single bolus TNK-t-PA was administered over 5 to 10 seconds with doses ranging from 5 to 50 mg.⁹ The agent was fibrin-specific (systemic fibrinogen and plasminogen level fell by only 3% and 13%, respectively, at 1 hour after bolus injection) with TIM1 grade 3 flow at 90 min of 59% (30 mg) and 64% (50 mg) and had a good safety profile. In the TIM1 10B study, a large phase II efficacy trial in 837 patients, a single 40 mg bolus of TNK-t-PA produced TIM1 grade 3 flow rates at 90 minutes that were identical to those seen with accelerated alteplase (63% in both groups).⁹ Furthermore, TIM1 frame counting in TIM1 10B was similar with 40 mg of TNK-t-PA and with accelerated alteplase (median frame counts 32 and 34, respectively). The best angiographic results were obtained with a dose/weight ratio of ± 0.5 mg/kg. The safety-evaluable cohort in ASSENT-I trial comprises 3325 patients with acute myocardial infarction. An intracranial haemorrhage rate of 0.73% was observed with the 30 mg dose of TNK-t-PA and 0.6% with the 40 mg dose.¹⁰ On the basis of the results of TIM1 10B and ASSENT-I, 16,500 patients with acute myocardial infarction were being randomized in a double-blind double-dummy ASSENT-2 trial and received "stepped" weight-adjusted TNK-t-PA (-0.5 mg/kg) dose or front-loaded alteplase (100 mg). All patients were on aspirin and intravenous heparin. The primary objective was to demonstrate equivalence in 30-day mortality between the two trial drugs. The 30-day mortality was 6.17% with TNK-t-PA and 6.15% with alteplase with an identical rate of intracranial bleeding (0.93% vs 0.94%). Other severe bleeding complications were less frequent in TNK-t-PA treated patients (0.83% vs 1.05%, $P = 0.151$) (data presented by F Van de Werf at the Convention of the American College of Cardiology, 1999).

Reteplase (r-PA, BM 06022, RapilysinTM, RetavaseTM, EkokinaseTM, Boehringer Mannheim) is a single chain non-glycosylated deletion mutant of wild-type human t-PA which contains the kringle 2 and the protease domain of human tPA but lacks its kringle 1 and the finger and growth factor domains (Fig. 4). Reteplase contains amino acids 1-3 and 176-527 of rt-PA (deletion of Val⁴-Glu¹⁷⁵). The Arg²⁷⁵-Ile²⁷⁵ plasmin cleave site is maintained.¹¹ The structural differences between reteplase and alteplase translate into a decreased fibrin binding but preferential activation of fibrin bound plas-

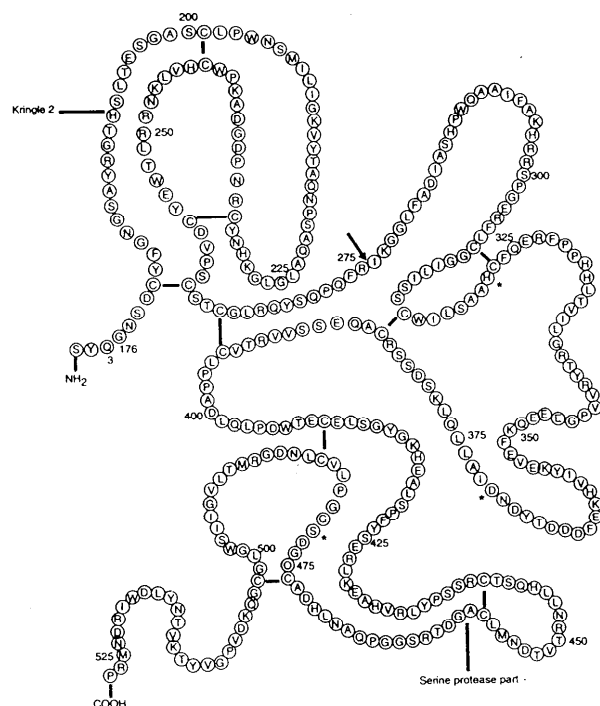


Fig. 4. Schematic representation of the primary structure of reteplase (amino acids Ser¹-Gln¹ and Gly¹⁷⁶-Pro⁵²⁷ of t-PA). The amino acids are represented by their single-letter symbols, black bars indicate disulfide bonds, and asterisks indicate active site residues in the protease part. The arrow indicates the plasmin cleavage site.

minogen and an extended half-life of reteplase compared to alteplase.

The active site in the proteinase domain of reteplase and of t-PA and their plasminogenolytic activity in the absence of a stimulator do not differ. However, the plasminogenolytic activity of reteplase in the presence of CNBr fragments of fibrinogen as a stimulator was found to be 4-fold lower as compared to t-PA, whereas the binding of reteplase to fibrin was 5-fold lower.^{11,12} These differences might possibly be due to the missing finger domain in reteplase. It is indeed known that fibrin binding is mediated through both the finger domain and the lysine-binding site in the kringle 2 domain of t-PA. Reteplase and t-PA are inhibited by PAI-1 to a similar degree. The affinity of reteplase for binding to endothelial cells and monocytes is reduced as compared to t-PA, probably as a consequence of deletion of the finger and epidermal growth factor domains in reteplase which seem to be involved in the interaction with endothelial cell receptors.¹³

Pharmacokinetic analysis of plasma activity in the rabbit revealed a half-life of 18.9 ± 1.5 min for reteplase and 2.1 ± 0.1 min for alteplase, with a 4.3-fold slower plasma clearance for reteplase than for alteplase.¹⁴ In healthy human volunteers¹⁵ and in patients with acute myocardial infarction¹⁶ an initial half-life of 14 to 18 min was observed for reteplase.

Different doses of reteplase in patients with acute myocardial infarction were evaluated in two open non-randomized pilot trials.^{17,18} In the randomized RAPID-I trial, two bolus doses of 10 MU reteplase appeared to achieve higher rates of early reperfusion than front-loaded alteplase.¹⁹ In the INJECT study, a double-blind randomized trial in 6010 patients with acute myocardial infarction, administration of two boluses of 10 MU reteplase given 30 min apart was compared with streptokinase (1.5 MU i.v. over 60 min).²⁰ Treatment could be started up to 12 hours after onset of symptoms. All patients received intravenous heparin for at least 24 hours and aspirin for several weeks. At one month, there were 270 deaths (9.0%) in the reteplase group and 285 deaths (9.5%) in the streptokinase group, a non-significant difference (95% CI 1.98 to 0.96). As the upper limit of the 90% CI for this difference is 0.71%, this result shows that reteplase is at least as effective as streptokinase. Bleeding events were similar in the two treatment groups (with reteplase 0.7%; with streptokinase 1%).

As compared with an accelerated infusion of alteplase in the Gusto-III trial, reteplase, although easier to administer, did not provide an additional 30-day survival benefit in the treatment of 15,059 patients with acute myocardial infarction.²¹ Other results, particularly for the combined end point of death or non-fatal disabling stroke, were remarkably similar for the two plasminogen activators.

Lanoteplase (nPA, BMS-200980, SUN9216, Bristol-Myers Squibb) is a deletion mutant of tissue type plasminogen activator. The finger-like fibronectin and epidermal growth factor (EGF) domains are deleted and Asn⁻¹¹⁷ is substituted by Gln⁻¹¹⁷ on kringle 1²² (Fig. 5). This point mutation eliminates an N-linked high mannose oligosaccharide carbohydrate side-chain present in the kringle 1 region of t-PA that is recognized by receptors on liver Kupffer cells.

In a rabbit model of pulmonary embolism lanoteplase had a four-fold longer circulating half-life than wild-type t-PA and caused more rapid thrombolysis without increased systemic fibrinogenolysis.^{22,23} In the dog, the hepatic clearance of lanoteplase was approximately six times slower than the clearance of wild-type t-PA (α -phase 8.9 ± 1.7 min versus 2.8 ± 0.6 min). An equal bolus of lanoteplase in dogs resulted in slightly more rapid coronary thrombolysis (copper-coil technique) than native t-PA of fresh thrombi (less than 30 minutes of coronary thrombosis) but was associated with a high reocclusion rate.²⁴ In human volunteers, the half-life for plasminogen activation was circa 3.7 ± 1.1 minutes.²⁵

A phase II controlled angiographic dose-ranging international trial of lanoteplase has been conducted (InTIME-I: Intravenous n-TPA for the treatment of early infarcting myocardium).²⁶ A total of 602 patients were randomized to either front-loaded alteplase (100 mg) or

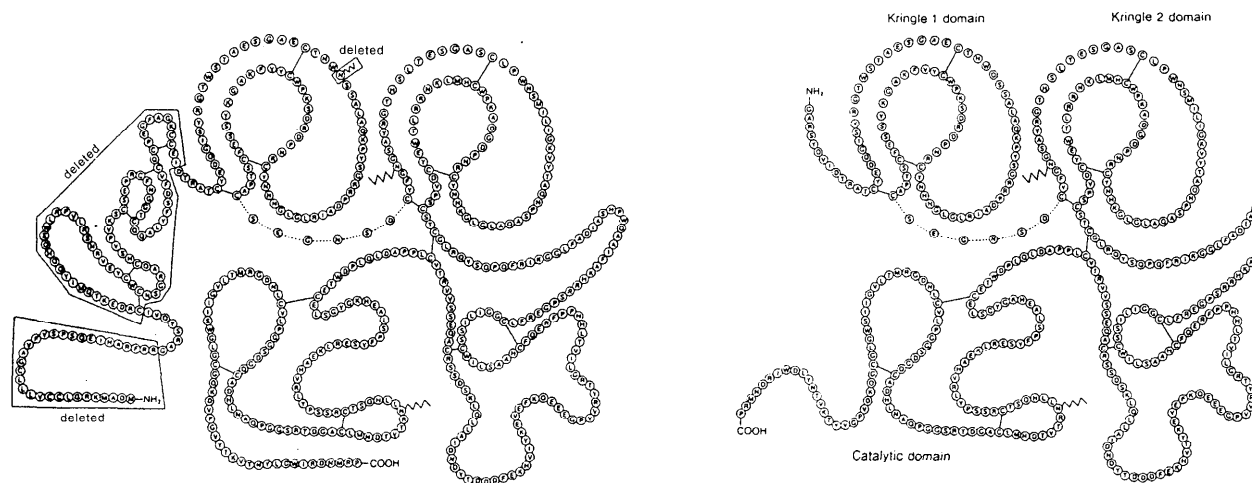


Fig. 5. Schematic representation of the primary structure of lanoteplase (nPA) (right), a deletion mutant of t-PA (left). The finger-like/broctectin and epidermal growth factor (EGF) domains are deleted and Asn¹¹⁷ is substituted by Gln¹¹⁷ in kringle 1

to one of four doses of lanoteplase administered as a weight-adjusted bolus of 15, 30, 60 or 120 KU/kg. The highest dose group of lanoteplase achieved patency TIM1 flow grade 2 or 3 at 90 minutes in 83% of patients, compared with 71% in the t-PA group ($P < 0.05$). Complete reperfusion (TIM1 flow grade 3) at 90 min, was achieved in 57% of the highest dose group of lanoteplase and in 46% of the alteplase group ($P = 0.11$). After 30 days, there was a trend toward fewer adverse outcome events (death, second non-fatal myocardial infarction, major bleeding, and heart failure) in the highest dose group of lanoteplase (9% versus 22% in the alteplase group). Lanoteplase will be compared with alteplase in a large mortality trial (InTIME-2), and the results should be available in early 1999.

YM866 (Pamiteplase, SolinaseTM, Yamanouchi Pharmaceuticals) is a glycoprotein composed of 445 amino acids. It is a modified t-PA, by deleting the amino acids residues 92 to 173 of kringle 1 and replacing the arginine at position 275 with glutamic acid.²⁷ In the presence of soluble fibrin, the plasminogen activation rate was 280-fold, whereas that of t-PA was increased only 24-fold.** In addition, the residence time of YM866 in the rat was 61 min versus 9 min for t-PA and the half-life (β phase) was 27 min versus 9 min for t-PA. In a coronary artery thrombosis model in dogs, the thrombolytic effect of an intravenous bolus injection of YM866 was dose dependent with a reperfusion rate 4 times higher than that of t-PA.²⁹ The specific activity of YM866 determined by the fibrin clot lysis time method calibrated with the international t-PA standard was 570,000 U/mg, a comparable value to that of t-PA (600,000 U/mg).

A study in healthy individuals confirmed the longer half-life of YM866.³⁰ The intracoronary artery injection of YM866 in patients with acute myocardial infarction

has confirmed the efficacy and safety of the agent.³¹ In a randomized double-blind trial in 229 patients with acute myocardial infarction, YM866 (intravenous bolus of 0.1 mg/kg) was compared to t-PA (14.4 mg/kg over 60 min). TIMI grade 3 was obtained 60 min after start of thrombolytic treatment in 50% of patients treated with YM866 and in 48% of the t-PA group.³² There was no significant decrease of fibrinogen after treatment with YM866. The authors concluded that the two drugs had the same thrombolytic effect while YM866 was associated with more fibrinogen sparing than t-PA. Large scale studies in patients with acute myocardial infarction have still to be conducted to demonstrate that treatment with YM866 is associated with the same or a lower mortality rate than t-PA and with less cerebral bleeding.

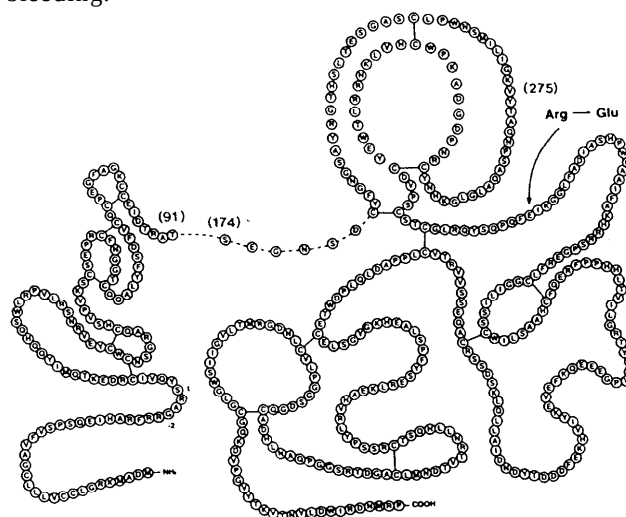


Fig. 6. Schematic representation of the primary structure of YM866, a mutant of t-PA with deletion kringle 1 domain and with point mutation (Arginine²⁷⁵ to glutamic acid) at the site of the kringle 2 domain linkage to the right chain.

Recombinant Unglycosylated and Glycosylated scu-PA (Pro-urokinase)

Scu-PA or pro-urokinase (pro-UK) is the single-chain zymogen of the serine protease urokinase (UK) (Fig. 6) or urokinase-type plasminogen activator (u-PA). Both the pro-UK zymogen and urokinase, the active two-chain enzyme, have been employed as fibrinolytic agents.

Saruplase (Rescueplase™, Grünenthal, Aachen) is the non-glycosylated form of scu-PA produced by recombinant technique in *E. coli*. The single peptide chain of 411 amino acids (MW 46.3 kDa) is identical to the natural scu-PA. PRIM1 is the first randomized double-blind trial comparing 80 mg unglycosylated recombinant scu-PA (saruplase), given as a 20-mg bolus followed by an infusion of 60 mg in 1 h, with standard dose streptokinase (SK) (1.5 million IU in 1 h).³³ A total of 401 patients with acute myocardial infarction of <4 hours in duration were randomized into the two treatment groups. Angiographic patency (TIMI 2 or 3) at 60 min was 72% for scu-PA and 48% for SK ($P < 0.001$), but at 90 min the differences became insignificant (71% and 64% for scu-PA and SK, respectively). A reocclusion rate at 24 to 36 h of only 1 of 108 restudied patients occurred in the unglycosylated scu-PA-treated patients, compared with 5 of 105 treated with SK, respectively. A major drop in fibrinogen occurred in both groups, being 86% and 94% for scu-PA and SK, respectively, but the transfusion requirements were significantly ($P < 0.01$) less in the scu-PA group (4.0% vs 11.3% for SK). The largest reported study with unglycosylated scu-PA is an open-label, multicentre study of 1698 patients treated within 6 hours of onset of symptoms of myocardial infarction.³⁴ The same 80 mg dosage scheduled used in the PRIM1 trial was administered, and most patients received a bolus of heparin prior to scu-PA infusion followed by a heparin infusion for 24 h. The major in-hospital clinical outcomes included mortality of 5.4%, reinfarction 3.8%, haemorrhagic stroke 0.5%, and transfusion required in 1.2% of the patients.

Recombinant glycosylated scu-PA (r-pro-UK; ABT-187, Prolyse™, Abbott) is a single-chain urokinase-type plasminogen activator which is purified to homogeneity from the culture medium of mouse hybridoma cells.³⁵ Compared to unglycosylated scu-PA, glycosylation of scu-PA results in greater fibrin specificity and stability at equivalent dosages.³⁶ Data have been accumulated from extensive preclinical studies which demonstrated the efficacy of glycosylated recombinant scu-PA as a potent thrombolytic agent. These data were derived from both *in vitro* clot lysis experiments in human plasma and a canine femoral artery thrombosis model. In the *in vitro* clot lysis system, with use of clots prepared from fresh-frozen human plasma, glycosylated recombinant scu-PA exhibited a lag phase to the onset of lysis and a concentration-dependent threshold effect due to the

presence of inhibitors. Effective clot lysis was achieved without degradation of the fibrinogen in the plasma. Over a dose range of 50,000 to 220,000 IU, the canine femoral artery thrombosis model showed a dose-dependent relationship for glycosylated recombinant scu-PA and effective clot lysis. The lytic activity of recombinant glycosylated scu-PA is significantly enhanced by the concomitant administration of heparin as adjunctive therapy. Fibrinogen, plasminogen and a₂-antiplasmin levels were unaltered or only minimally so during the many thrombolytic studies carried out in the femoral artery occlusion model.³⁷

Two pilot studies evaluated the safety and efficacy of recombinant glycosylated scu-PA in patients with acute myocardial infarction.^{37,38} The first was an open, multicentre study comparing two different doses of recombinant glycosylated scu-PA, 60 mg and 80 mg; each dose was given as a bolus + infusion over 60 or 90 min ($n = 46$). The second study was a double-blind, multicentre trial in which 85 patients received either a priming bolus dose of 250,000 IU of recombinant urokinase followed immediately by 60 mg of recombinant glycosylated scu-PA or a placebo priming agent followed immediately by 80 mg recombinant glycosylated scu-PA. In study 1, the 90-min patency rate (TIMI grade 2 or 3) was 66.7% in 21 patients receiving 60 mg over 60 min and 72.2% in 18 patients receiving 60 mg over 90 min. In study 2, the 90-min patency rates were 45.5% in the 60 mg primed group infused over 60 min (11 patients) and 80.8% in the primed group infused over 90 min (26 patients). In the 80 mg unprimed group, the 90 min patency rates were 100% in the 6 patients infused

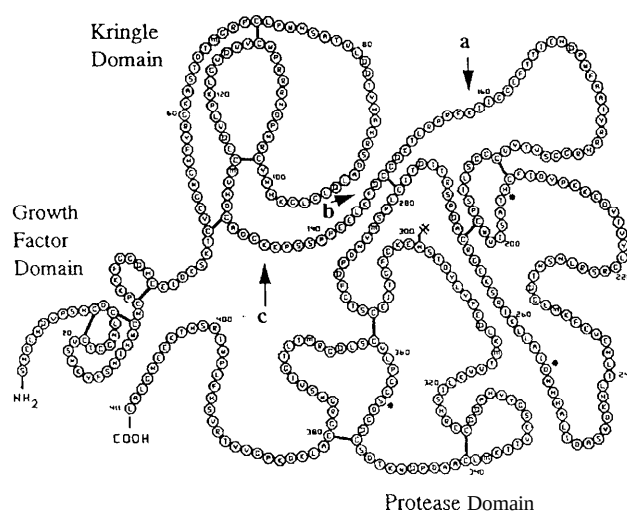


Fig. 7. Primary structure of r-pro-UK (ABT-187). Asterisks mark the locations of the amino acids involving the active site of UK (His-204, Asp-255 and Ser-356). Arrows mark the sites of cleavage by proteolytic enzymes. Cleavage at sites a (Lys¹⁵⁸-Ile¹⁵⁹) produces active two-chain-molecular-weight UK; cleavage at sites a and b, or a and c generate low-molecular-weight UK. Cleavage at site b or c generates low-molecular-weight r-pro-UK molecules. The symbol at residue Asn-302 represents the site of N-linked glycosylation.

over 60 min and 69.2% in the 26 patients infused over 90 min. The rate of angiographic reocclusion in those who had no coronary intervention was 1.4% during the 18 h following the acute phase. Only 4.6% of patients experienced severe bleeding complications, with no patient developing brain hemorrhage.

Staphylokinase

Staphylokinase, a plasminogen activator produced by certain strains of *Staphylococcus aureus*, was shown to have profibrinolytic properties more than 4 decades ago.^{39,40} Natural staphylokinase has been purified from *Staphylococcus aureus* strains which were transformed with bacteriophages containing the staphylokinase gene, or which had undergone lysogenic conversion to staphylokinase production.^{41,42} In addition, the staphylokinase gene has been cloned from the bacteriophages sakC⁴³ and sak42D⁴⁴ as well as from the genomic DNA (sakSTAR) of a lysogenic *S. aureus* strain.⁴²

The staphylokinase gene encodes a protein of 163 amino acids with amino acid 28 corresponding to the NH₂-terminal residue of the mature protein, which consists of 136 amino acids in a single polypeptide chain without disulfide bridges. The mature proteins SakSTAR, SakoC and Sak42D differ in only three amino acids; amino acid 34 is Ser in SakSTAR but Gly in SakoC and Sak42D, amino acid 36 is Gly in SakSTAR and in SakoC, but Arg in Sak42D, and amino acid 43 is His in SakSTAR and in SakoC, but Arg in Sak42D.⁴⁵⁻⁴⁷ In purified preparations, different molecular forms of staphylokinase with slightly different molecular weight and isoelectric points have been observed; molecular forms have been characterized lacking the 6 or the 10 NH₂-terminal amino acids.^{42,46,47}

Staphylokinase forms a 1:1 stoichiometric complex with plasmin(ogen).^{48,49} It is not an enzyme, and generation of an active site in its equimolar complex with plasminogen requires conversion of plasminogen to plasmin. Thus, the plasmin.staphylokinase complex is the active enzyme.^{50,51} This is in contrast with streptokinase which produces a complex with plasminogen which exposes the active site in the plasminogen moiety without proteolytic cleavage.⁵² Kinetic data suggest the following mechanism for plasminogen activation in a buffer milieu.

Plasminogen (P) and staphylokinase (S) produce an inactive 1:1 stoichiometric complex (P.S) which does not activate plasminogen. The activation reaction appears to be initiated by trace amounts of plasmin (p) which generates active plasmin.staphylokinase complex (p.S).⁵¹ In mixtures with excess plasminogen over staphylokinase, generated p.S converts excess plasminogen to plasmin. In addition, kinetic analysis has revealed that generated p.S converts P.S to p.S, thus representing a potential positive feed-back mechanism.⁵³ However, the

main pathway for plasmin generation in the above scheme appears to be via activation of P by p.S formed by binding of S to p. This is supported by binding data obtained with fluorescently-labelled staphylokinase, showing that it has a much higher affinity for plasmin than for native plasminogen.⁵⁴

In patients with acute myocardial infarction treated with an intravenous infusion of 10 mg staphylokinase (SakSTAR) over 30 min, the concentration of staphylokinase-related antigen in blood at the end of the infusion increased to between 0.9 and 1.7 µg/ml. The post-infusion disappearance of staphylokinase-related antigen from plasma occurred in a biphasic manner with a half-life α of 6.3 min and half-life β of 37 min, corresponding to a plasma clearance of 270 ml/min.⁵⁵ In the STAR trial, SakSTAR antigen levels in 25 patients with acute myocardial infarction receiving 10 mg i.v. over 30 min were 0.56 ± 0.06 µg/ml at 25 min and 0.16 ± 0.04 µg/ml at 90 min, with corresponding levels of 1.9 ± 0.22 µg/ml and 0.42 ± 0.06 µg/ml in 23 patients receiving 20 mg SakSTAR over 30 min.⁵⁶

The rather low-grade antigenicity of staphylokinase, as suggested by early dog and baboon experiments, unfortunately is not extended to patients. The vast majority of patients with either myocardial infarction^{55,56} or peripheral arterial occlusion⁵⁷ developed neutralizing antibodies to SakSTAR, albeit after a long lag phase of 7 to 12 days, that remained elevated well above pretreatment levels for several months after administration.⁵⁸ However, the titres of preformed anti-SakSTAR antibodies in the general population appeared to be lower than those of anti-streptokinase antibodies,⁵⁹ and even systemic *S. aureus* infections failed to induce SakSTAR-neutralizing antibodies in most patients,⁵⁷ possibly reflecting the low proportion of *S. aureus* strains that produce staphylokinase. The boost of neutralizing antibody titres upon infusion of SakSTAR, however, predicts therapeutic refractoriness on repeated administration.

In the first pilot recanalization study, patients with acute myocardial infarction were given 10 mg i.v. SakSTAR as a 1 mg bolus followed by infusion of 9 mg over 30 min.⁵⁸ Patients randomized to i.v. SakSTAR were given 10 mg over 30 min in the first half of the study and, following a prospectively planned interim analysis, 20 mg over 30 min in the second half, always with an initial 10% bolus. At 90 minutes TIM1 grade 3 patency was obtained in 58% of patients treated with t-PA and in 62% of patients treated with staphylokinase. In a phased, angiographically controlled pilot study on bolus Sak42D infusion for coronary thrombolysis, 20 mg Sak42D was given over 5 min at study entry, with a second bolus of 10 mg over 5 min at 60 min if angiography showed TIM1 perfusion grade 0, 1 or 2.⁶⁰ The encouraging experience obtained in this pilot study inspired a multicentre

randomized trial in patients with evolving myocardial infarction, comparing accelerated rt-PA with a double bolus of 15 mg Sak42D given 30 min apart.

In a pilot study in patients with peripheral arterial occlusion, intra-arterial catheter-directed SakSTAR was given as a bolus of 1 mg, followed by a continuous infusion of 0.5 mg per hour, or as a 2 mg bolus followed by an infusion of 1 mg per hour, together with heparin. Complete recanalization was obtained in 83% of the patients after 7.0 ± 0.7 mg SakSTAR infused over 8.7 ± 1.0 h.⁵⁹

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