Thrombosis, the blockage of blood vessels with clots, can lead to acute myocardial infarction and ischemic stroke, both leading causes of death. Other than surgical interventions to remove or by pass the blockage, or the generation of collateral vessels to provide a new blood supply, the only treatment available is the administration of thrombolytic agents to dissolve the blood clot. In this review, Désiré Collen considers the properties and characteristics of staphylokinase that make it the thrombolytic agent of choice.

Staphylokinase: a potent, uniquely fibrin-selective thrombolytic agent

DÉSIRÉ COLLEN

Acute myocardial infarction and ischemic stroke are the two main causes of death and disability in our Western societies. Thrombolytic therapy of these diseases is based on the premise that their proximal cause is thrombosis, triggered by rupture of an atheromatous plaque in the wall of critically situated blood vessels. Occlusive thrombosis results in loss of blood flow to vital organs producing local oxygen deprivation, cell necrosis and loss of organ function. The hypothesis underlying thrombolytic therapy of thromboembolic disease is that early and sustained recanalization prevents cell death, reduces infarct size, preserves organ function, and reduces early and late mortality. Thrombolysis consists of the pharmacological dissolution of the blood clot, by intravenous infusion of plasminogen activators that activate the fibrinolytic system (Fig. 1). The fibrinolytic system includes a proenzyme, plasminogen, which is converted by plasminogen activators to the active enzyme plasmin, which in turn digests fibrin to soluble degradation products.

Inhibition of the fibrinolytic system takes place at the level of the plasminogen activators (mainly by plasminogen activator inhibitor-1) and of plasmin (mainly by α_2-antiplasmin)[1]. Thrombolytic agents that are either approved or under clinical investigation in patients with acute myocardial infarction include streptokinase, recombinant tissue-type plasminogen activator (rt-PA or alteplase), rt-PA derivatives such as reteplase and TNK-rtPA, anisoylated plasminogen-streptokinase activator complex (APSAC or anistreplase), two-chain urokinase-type plasminogen activator (tcu-PA or urokinase), recombinant single-chain u-PA (scu-PA, pro-u-PA or prourokinase), and recombinant staphylokinase and derivatives. Recently, rt-PA was also approved for treatment of ischemic stroke. The beneficial effects of thrombolytic therapy in acute myocardial infarction have been well established in controlled clinical trials (Table 1) and it has become routine treatment[2]. Although it is given to more than 500,000 patients per year worldwide, while at least three times that number could benefit from this treatment.

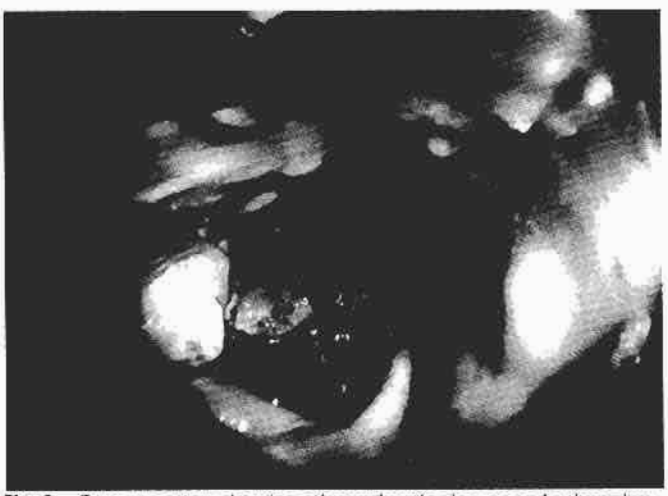


Fig. 1 Coronary artery showing atherosclerotic plagues and a thrombus.

Although all thrombolytic agents act by converting plasminogen to plasmin, which dissolves the fibrin of blood clots, they are not all equal. Indeed, physiological fibrinolysis is regulated by specific molecular interactions between its main components tissue-type plasminogen activator (t-PA), plasminogen and fibrin by which plasminogen is preferentially activated at the fibrin surface, where generated plasmin is protected from rapid inhibition by α_2-antiplasmin and thus may efficiently degrade the clot[1] (Fig. 2). Thus fibrin-selective agents (rt-PA and derivatives, staphylokinase and derivatives and to a lesser extent scu-PA) that digest the clot in the absence of systemic plasminogen activation are distinguished from non fibrin-selective agents (streptokinase, tcu-PA and APSAC), which activate systemic and fibrin-bound plasminogen indiscriminately. Non fibrin-selective agents are less efficient for clot dissolution and cause a systemic generation of plasmin, depletion of α_2-antiplasmin and degradation of coagulation factors, (this however protects against reocclusion of the infarct-related artery)[1]. In contrast to non fibrin-selective agents, fibrin-selective agents require conjunctive use of heparin anticoagulation, as established in several mechanistic studies in experimental animals and patients[4] and confirmed by meta-analysis of mortality in over 100,000 patients[5].

Two megatrials, in groups of over 10,000 patients with acute myocardial infarction each, initially compared the effects of streptokinase and rt-PA (in the absence of intravenous heparin) on mortality and found no difference. However, the *Global Utilisation of Streptokinase and rt-PA for Occluded* coronary arteries (GUSTO) trial conclusively demonstrated that fibrin-selective rt-PA combined with intravenous heparin is superior to non fibrin-selective streptokinase for early mortality reduction (7.3% with streptokinase versus 6.3% with rt-PA, $p = 0.001$)[6]. A recent meta-analysis of all megatrials concluded differently[7], but made the fundamental mistake of pooling the rt-PA and the rt-PA plus streptokinase groups of GUSTO, which however constitute fibrin-selective and non fibrin-selective thrombolytic strategies with different mortality reductions. Post-hoc analysis of GUSTO results indicated that the difference in mortality persisted after one year[8] and that patients

who were at higher risk derived a greater absolute benefit from treatment with rt-PA and intravenous heparin than with streptokinase[9]. Thus, as discussed in more detail elsewhere[5], fibrin-selectivity is a desirable property of thrombolytic agents.

Thrombolytic agents can also be distinguished on the basis of their origin, in autologous substances derived from the human body (rt-PA and derivatives, u-PA and derivatives) or heterologous substances of prokaryotic origin (streptokinase, anistreplase, staphylokinase). Although the former are non-immunogenic and can thus be used repeatedly, the latter induce neutralizing antibody formation and are restricted to single use.

All available thrombolytic agents suffer significant shortcomings including large therapeutic doses, limited efficacy, reocclusion and bleeding complications. Thus rt-PA, the most effective agent presently available, will produce functional recanalization of the occluded coronary artery in approximately 55 percent of patients, recanalization requiring 45 minutes or more, whereas reocclusion occurs in 5 to 10 percent and cerebral bleeding in 0.5 to 1.0 percent of patients. Thus there is a need for more efficient, safe and relatively inexpensive thrombolytic agents.

This review summarizes the present knowledge of the pharmacodynamic, thrombolytic and immunogenic properties of

Box Mechanisms of plasminogen (Plg) activation by staphylokinase (Sak)[a]

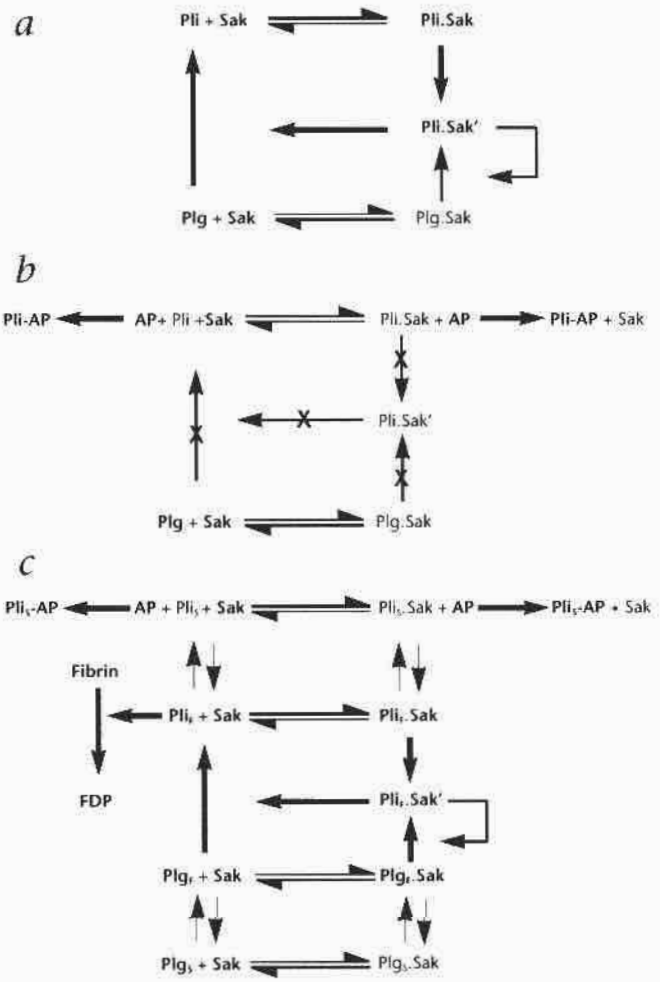
In a buffer milieu (Fig. *a*), the activation is primed by traces (as little as 3 ppm may initiate the process) of plasmin (Pli) which reacts with Sak and processes it to Sak' by hydrolysis of the Lys10-Lys11 peptide bond[23], forming an equimolar Pli.Sak' complex[24]. Sak has a much lower affinity for native Plg than for Pli (binding constant of less than 8 μM as compared to 50 nM) or for Plg bound to partially degraded fibrin[25]. Thus, in solution, Sak primarily reacts with Pli to generate Pli.Sak', which converts Plg to Pli, whereas on the fibrin surface, Sak reacts with fibrin bound Pli to generate Pli.Sak', which converts fibrin bound Plg.Sak to Pli.Sak' and fibrin-bound Plg to Pli. Whether Pli processes free Sak or Sak complexed with Plg or Pli, or whether Pli.Sak processes free or complexed Sak is presently unknown. The Pli.Sak' complex converts Plg to Pli and Plg.Sak or Plg.Sak' to Pli.Sak'. Activation of Plg by the preformed Pli.Sak' complex obeys Michaelis-Menten kinetics with K_m = 6.7 μM and k_2 = 1.8 s^{-1} [26]. Sak does not bind to fibrin, and fibrin does not markedly stimulate the initial rate of Plg activation by Sak[29]. Finally, all Plg is converted to Pli and all Sak to Sak'.

In a plasma milieu in the absence of fibrin (Fig. *b*), α_2-antiplasmin (AP) eliminates free plasmin and thereby prevents processing of Sak to Sak', generation of Pli.Sak' and conversion of Plg to Pli. Any generated Pli.Sak or Pli.Sak' complex is neutralized by α_2-antiplasmin, producing inactive plasmin-α_2-antiplasmin (Pli-AP) complex, and dissociation of functionally active Sak or Sak'[26].

In a plasma milieu in the presence of fibrin (Fig. *c*), traces of Pli (generated by physiological plasminogen activation ?) bound to fibrin (Pli_f) via its lysine binding site, are protected against rapid inhibition by α_2-antiplasmin (AP). Sak, which binds with greater affinity to fibrin-bound Pli_f and Plg_f than to circulating Plg_s, is locally processed and generates Pli_f.Sak' complex, which converts fibrin-bound Plg_f to Pli_f and Plg_f.Sak to Pli_f.Sak'. Pli_f.Sak' released from the degraded fibrin clot, now Pli_s.Sak', is rapidly neutralized by α_2-antiplasmin, and functionally active Sak' is released and is recycled to fibrin-bound Pli_f and Plg_f.

Sak is comparably active towards platelet-poor (unretracted) and platelet-rich (retracted) clots, whereas streptokinase is only able to dissolve platelet-poor clots[27]. This differential sensitivity of platelet-rich clots to fibrin-selective Sak versus non fibrin-selective streptokinase probably results from alteration of the AP to Plg ratio in the clot during retraction. Indeed, Plg is weakly associated with fibrin via its lysine-binding site and may be extruded, whereas AP is crosslinked to fibrin by factor XIIIa. Thus, upon retraction the ratio of Plg to AP in the clot decreases. Retracted blood clots have previously been shown to be more sensitive to lysis with the fibrin-selective plasminogen activator t-PA, than with the non fibrin-selective agents streptokinase and urokinase[28]. This phenomenon was explained by extensive systemic Plg activation with non fibrin-selective agents, which precludes recruitment of Plg from the surrounding plasma and thereby results in reduced clot lysis. These properties may be clinically relevant because the high platelet content of a coronary thrombus, together with retraction and ageing, are believed to significantly limit the thrombolytic efficacy of conventional non fibrin-selective agents.

Thus, fibrin orchestrates its plasminogen-mediated destruction by Sak, by priming it with bound Pli (Pli_f), by accelerating it as a scaffold for facilitated generation of protected Pli_f.Sak' and Pli_f, and by letting AP extinguish it after its removal. The thermodynamics of the equilibria, the kinetics of the reactions and the pharmacokinetics of the reactants governing these mechanisms of fibrin-selectivity are such that, at therapeutic doses of staphylokinase of up to 40 mg given over 30 minutes to patients with acute myocardial infarction, no significant plasminemia is observed, as evidenced by the absence of systemic plasminogen activation, AP consumption and fibrinogen breakdown.



[a] In this box, the following abbreviations are used: Sak: mature staphylokinase with NH_2-terminal sequence Ser-Ser-Ser; Sak': processed Sak with NH_2-terminal Lys-Gly-Ala- generated by plasmin mediated hydrolysis of the Lys10-Lys11 peptide bond in mature Sak; Plg: native plasminogen with NH_2-terminal Glu; Pli: plasmin; AP: α_2-antiplasmin; Plg.Sak: stoichiometric 1:1 complex of Plg with Sak; Pli.Sak: complex of Pli with Sak; Pli-Sak': complex of Pli with processed Sak, endowed with plasminogen activating activity; Pli-Ap: complex of Pli with AP; subscript F: fibrin-bound; subscript S: soluble (e.g. Pli_s, Pli_f, Pli_s.Sak', Pli_f.Sak')

staphylokinase, an "old" substance with "new" therapeutic perspectives, as recently inferred from its potency and unique fibrin-selectivity. The name staphylokinase was coined in 1948(ref. 10, 11), analogous to the earlier designation of streptokinase for the plasminogen-dependent fibrinolytic activity of streptococci[12]. The thrombolytic potency of staphylokinase was studied in 1964 and in 1985 in dogs, but it was found to be characterized by poor thrombolytic efficacy, severe bleeding and complete fibrinogen degradation[13,14]. In retrospect, however, these *in vivo* studies have been misleading because the canine fibrinolytic system is unusually sensitive to systemic activation with staphylokinase[15]. The mechanism of activation of plasminogen by staphylokinase was found to bear similarities to that of streptokinase but also to differ in some essential aspects, for example, the molecule is endowed with a unique mechanism of fibrin-selectivity in a human plasma milieu[3,16]. In line with initial biochemical and subsequent experimental animal studies, recent administration of staphylokinase to over 300 patients with thromboembolic disease has revealed that it holds promise for potent and highly fibrin-selective thrombolytic therapy.

Structure of staphylokinase

The *staphylokinase* gene has been cloned from the serotype B bacteriophage *sak*øC[17], from the serotype F bacteriophage *sak*42D[18] and from the genomic DNA (*sak*STAR) of a staphylokinase secreting *Staphylococcus aureus* strain[19].

The *staphylokinase* gene encodes a protein of 163 amino acids, which is processed to a mature protein of 136 amino acids, consisting of a single polypeptide chain without disulfide bridges[18,20,21] (Fig. 3). The tertiary structure has recently been elucidated[22]; staphylokinase is an ellipsoidal molecule with principal axes of 54 Å, 42 Å and 30 Å (Fig. 4).

Functional properties and mechanisms of fibrin-selectivity

Like streptokinase, staphylokinase is not an enzyme but it forms a 1:1 stoichiometric complex with plasmin(ogen) that activates other plasminogen molecules. In a human plasma milieu, thrombolysis with streptokinase is highly non fibrin-selective, whereas thrombolysis with staphylokinase is highly fibrin-selective.

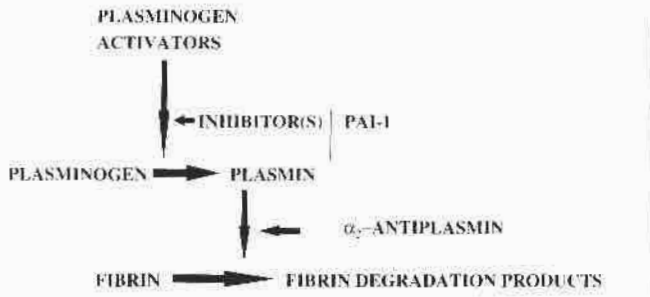


Fig. 2 Schematic representation of the fibrinolytic system. The proenzyme plasminogen is activated to the active enzyme plasmin by plasminogen activators. Plasmin degrades fibrin into soluble fibrin degradation products. Inhibition of the fibrinolytic system may occur at the level of the plasminogen activators by plasminogen activator inhibitors (PAI) or at the level of plasmin, mainly by α_2-antiplasmin. SK indicates streptokinase.

When streptokinase is added to human plasma containing a fibrin clot, it will complex with plasminogen (both at the fibrin clot surface and in solution). This complex, without further processing, is a potent activator of other plasminogen molecules and is resistant to all proteinase inhibitors in plasma. As a consequence, explosive plasminogen activation occurs in plasma, leading to: 1) plasminogen steal from the clot surface, thereby reducing the thrombolytic efficacy and 2) excessive systemic plasmin generation, leading to α_2-antiplasmin depletion and fibrinogen breakdown by residual plasmin.

When staphylokinase is added to human plasma containing a fibrin clot, it will react poorly with plasminogen in plasma, but will react with high affinity with traces of plasmin at the clot surface, becoming a plasmin.staphylokinase complex which efficiently activates plasminogen to plasmin at the clot surface. Plasmin.staphylokinase and plasmin bound to fibrin are protected from inhibition by α_2-antiplasmin. Plasmin.staphylokinase complex or plasmin, liberated from the clot or generated in plasma, are however rapidly inhibited by α_2-antiplasmin, thereby confining the process of plasminogen activation to the thrombus, preventing excessive plasmin generation, α_2-antiplasmin depletion and fibrinogen degradation in plasma. (The biochemical pathways governing these fibrin-selective interactions are summarized in Box; refs. 23-28).

The fibrin-selectivity of staphylokinase in a human plasma milieu is due to rapid inhibition of generated plasmin.staphylokinase complex by α_2-antiplasmin in plasma[26,29] and by a more than 100-fold reduction in the rate of inhibition at the fibrin surface[30], which allows preferential plasminogen activation at the fibrin clot surface. In the absence of fibrin, the plasmin.staphylokinase complex is neutralized by α_2-antiplasmin and no significant plasminogen activation occurs[31]. In the presence of fibrin, bound plasmin.staphylokinase complex is generated at the clot surface, its inhibition by α_2-antiplasmin is markedly delayed and efficient plasminogen activation occurs.

These molecular interactions between

Table 1 Randomized placebo-controlled trials of intravenous thrombolytic therapy in 500 patients with acute myocardial infarction

Trial	Thrombolytic agent	No. of patients	Short-term mortality Treatment %	Short-term mortality Control %	Follow-up interval days
ISAM	SK	7,741	6.3	7.1	21
GISSI	SK	11,712	10.7	13.0	21
ISIS-2	SK	17,187	9.1	11.8	35
ASSET	alteplase	5,011	7.2	9.8	30
ECSG-5	alteplase	721	2.8	5.7	14
AIMS	anistreplase	1,004	6.4	12.2	30
LATE	alteplase	5,711	8.9	10.3	35
EMERAS[a]	SK	4,534	13.2	14.4	35

SK: streptokinase; alteplase: tissue-type plasminogen activator; anistreplase: anisoylated plasminogen streptokinase activator complex (APSAC).
[a]86% of patients randomized 7 to 24 hours after onset of pain.
The table (reproduced from ref. 2) summarizes results of all studies in patients with acute myocardial infarction which randomized more than 500 patients to either placebo (control) or thrombolytic agent (treatment). Short-term mortality (after a follow up interval of 14 to 35 days) was 20 to 25 percent lower with thrombolytic therapy than in the control group, when initiated within 6 hours after the onset of symptoms.
This information constitutes the data base for the present routine application of thrombolytic therapy in acute myocardial infarction. It should be noted that because of the variability in patient selection, adjunctive use of antithrombotic agents and mechanical interventions, these results are not suited for comparison of treatment related mortalities among agents or trials.

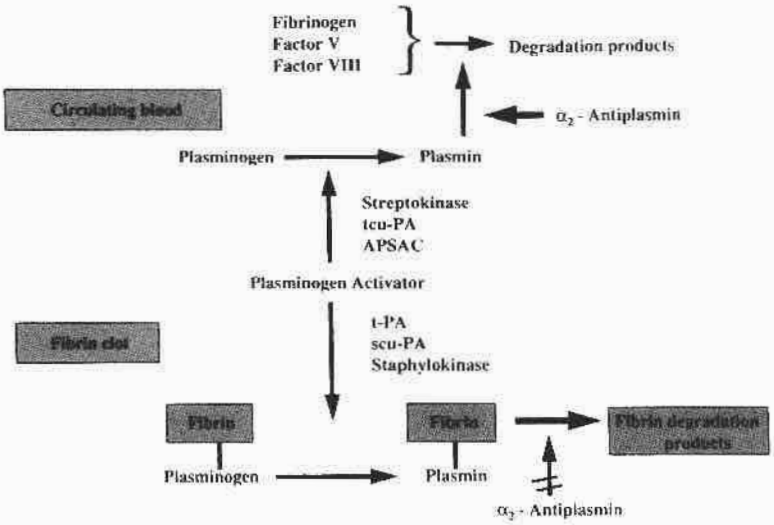


Fig. 3 Molecular interactions determining the fibrin-selectivity of plasminogen activators. Non fibrin-selective plasminogen activators (streptokinase, tcu-PA, APSAC) activate both plasminogen in the fluid phase and fibrin-associated plasminogen. Fibrin-selective plasminogen activators (t-PA, scu-PA and staphylokinase) preferentially activate fibrin-associated plasminogen.

staphylokinase, plasminogen, plasmin, α_2-antiplasmin and fibrin endow the molecule with a unique mechanism of fibrin-selectivity in a plasma milieu[5,10]. Fibrin-selectivity improves therapeutic efficacy by avoiding systemic plasminogen activation with "plasminogen steal" and plasmin-mediated prothrombotic effects, and might improve safety by avoiding the systemic fibrinolytic state. These postulated advantages however require clinical validation.

Species variability in fibrinolytic and thrombolytic response

The plasma fibrinolytic system in baboons, rabbits and hamsters was found to react to staphylokinase in the same way as the human system; the rat and bovine systems appeared to be very resistant, whereas the dog system was very sensitive, but not fibrin-selective[12].The reactivity of staphylokinase with human and canine plasminogen explains its high potency for clot lysis in these species, whereas the 10-fold lower reactivity of the canine plasmin.staphylokinase complex with canine α_2-antiplasmin may explain its markedly lower fibrin-specificity in the dog[13]. Because of the unusually high sensitivity of the canine plasma fibrinolytic system to non fibrin-selective activation with staphylokinase, the choice of the canine species for the initial *in vivo* evaluation of staphylokinase[11,14] in retrospect has produced misleading conclusions.

In hamsters with a pulmonary embolus consisting of a platelet-poor, a platelet-rich (300,000 platelets/μl), or a platelet-enriched (1,500,000 platelets/μl) human plasma clot, the relative thrombolytic potencies of staphylokinase and streptokinase, on a weight base, were comparable in the platelet-poor and in the platelet-rich clot model; but staphylokinase was 5-fold more potent than streptokinase in the platelet-enriched clot model[33]. In baboons, intravenous streptokinase or staphylokinase induced a similar dose-dependent fibrin-specific lysis of a ^{125}I-fibrin labeled autologous jugular vein blood clot, but recanalization with staphylokinase of a platelet-rich arterial eversion graft thrombus was more frequent and more persistent than with streptokinase[34].

In aggregate, these data confirm and establish that staphylokinase is a potent thrombolytic agent *in vivo* and that staphylokinase may be more potent than streptokinase for the dissolution of platelet-rich clots, such as those observed in the coronary arteries of patients with acute myocardial infarction.

Pharmacokinetics

In five patients with acute myocardial infarction treated with an intravenous infusion of 10 mg staphylokinase (SakSTAR) over 30 minutes, the concentration of staphylokinase-related antigen in blood at the end of the infusion ranged between 0.9 and 1.7 μg/ml. The post-infusion disappearance of staphylokinase-related antigen from plasma occurred in a biphasic mode with a t1/2α of 6.3 min (mean ± SD) and a t1/2ß of 37 min, corresponding to a plasma clearance of 270 ml/min[35].

Thrombolytic properties in patients

The thrombolytic potential of staphylokinase has been evaluated in patients with acute myocardial infarction[35–39] and with peripheral arterial occlusion[40].

Acute myocardial infarction

In two small pilot studies, 10 patients with coronary artery occlusion confirmed by angiography were treated by intravenous administration of 10 mg staphylokinase (SakSTAR), given as a 1 mg bolus and infusion of 9 mg over 30 minutes in conjunction with aspirin and heparin[15,16]. Complete arterial recanalization was obtained in eight patients and partial recanalization in one patient, with a mean time delay to reperfusion of about 20 minutes. Plasma levels of fibrinogen, plasminogen and α_2-antiplasmin remained unchanged, confirming the high fibrin-specificity of staphylokinase in man.

In an open randomized multicenter trial, staphylokinase (SakSTAR) was compared to accelerated weight-adjusted rt-PA in 100 patients with acute myocardial infarction[17]. Twenty-five patients received 10 mg intravenously over 30 minutes and 23 patients were given 20 mg over 30 minutes. At 90 minutes complete arterial recanalization was obtained in 58% of patients treated with rt-PA and in 62% of patients treated with staphylokinase (in 50% of patients receiving 10 mg and in 74% receiving 20 mg). Staphylokinase was highly fibrin-specific, as revealed by virtually unaltered levels of plasma fibrinogen, plasminogen and α_2-antiplasmin, whereas rt-PA caused a 30% drop in fibrinogen and a 60% decrease in plasminogen and α_2-

Fig. 4 Tertiary structure of staphylokinase (reprinted with permission from ref 22).View perpendicular to the β-sheets with the five-stranded β-sheet colored yellow, the two-stranded β-sheet blue and the α-helix red.

antiplasmin levels. No strokes, allergic reactions or other side effects were recorded[37]. Administration of 40 mg staphylokinase in five additional patients also did not produce detectable fibrinogen breakdown.

A pilot study of bolus staphylokinase infusion with aspirin and intravenous heparin was performed in 13 patients with evolving transmural myocardial infarction[38]. The first patient presenting with a large anterior myocardial infarction and treated with a double bolus of 2 times 20 mg given 15 minutes apart, however, developed a non-fatal but moderately disabling intracerebral hemorrhage. Therefore, in the next 12 patients, phased bolus injections were given under angiographic control. At entry 20 mg staphylokinase (Sak42D) was injected over 5 minutes and angiography was performed at 60 minutes, with an additional dose of 10 mg given if recanalization of the infarct-related artery was incomplete. Complete recanalization at 60 minutes was obtained in 7 patients (58%). Administration of a second bolus (10 mg over 5 minutes) to the remaining five patients, resulted in complete reflow in three of those at 90 minutes yielding an overall reflow rate of 83% at 90 minutes[38].

On the basis of these findings, a comparative trial of double bolus staphylokinase (Sak42D) of 15 mg each given 30 minutes apart (50 patients) versus front loaded rt-PA (52 patients) was carried out in 102 patients[39]. Complete reflow at 90 minutes was obtained in 68% of patients treated with staphylokinase and in 57% of patients given rt-PA (p = NS). Administration of Sak42D was fibrin-selective and did not cause allergic reactions, but the majority of the patients developed neutralizing IgG after 2 weeks.

These pilot clinical studies suggest that intravenous staphylokinase, combined with heparin and aspirin, is a potent, rapidly acting and highly fibrin-selective thrombolytic agent in patients with acute myocardial infarction.

Peripheral arterial occlusion

Thirty patients with angiographically confirmed peripheral arterial occlusion were treated with intra-arterial staphylokinase (SakSTAR), given as a 1 mg bolus followed by infusion of 0.5 mg per hour in 20 patients or as a 2 mg bolus followed by an infusion of 1 mg per hour in another 10 patients, all receiving heparin[40]. Recanalization was complete in 25 patients (83%), partial in two and absent in three patients. Two major hemorrhagic complications occurred, including one fatal hemorrhagic stroke. No severe allergic reactions were observed nor any morbidity relating to the intra-arterial use of staphylokinase. Antibody titers neutralizing ≥5 μg staphylokinase per ml plasma occurred after a lag phase of 1 to 2 weeks in 80 percent of the patients.

Immunogenicity

Levels of preformed anti-staphylokinase antibodies in the general population are lower than those of anti-streptokinase antibodies[41], whereas the current clinical experience in over 300 patients suggests that major allergic reactions to Sak are rare. However, most patients develop high titers of neutralizing specific IgG after infusion of staphylokinase, which would predict therapeutic refractoriness upon repeated administration.

In the STAR trial, in 48 patients with acute myocardial infarction, staphylokinase neutralizing activity levels were low at baseline (0.07 ± 0.01 μg/ml) and during the first week after staphylokinase administration (1.5 ± 0.39 μg/ml), but increased substantially from the second week on (32 ± 7.1 μg/ml)[37]. Also in patients with peripheral arterial occlusion, intra-arterial administration of staphylokinase elicited high levels of staphylokinase-neutralizing antibodies, which remained high for several months[40].

Efforts are presently being undertaken to reduce the immunogenicity of staphylokinase by site-directed mutagenesis. Wild-type staphylokinase (SakSTAR variant) was found to contain three non-overlapping immunodominant epitopes, at least two of which could be eliminated, albeit with partial inactivation of the molecule, by site directed substitution of clusters of two or three charged amino acids with alanine[42]. Two variants were identified, SakSTAR.M38 (with K35, E38, K74, E75 and R77 substituted with A) and SakSTAR.M89 (with K74, E75, R77, E80 and D82 substituted with A), that did not recognize approximately one third of the antibodies elicited in patients by treatment with wild-type SakSTAR[42]. In rabbits[43] and baboons[44], these variants elicited markedly less circulating neutralizing antibodies and significantly less resistance to repeat thrombolysis than wild-type SakSTAR. In patients with peripheral arterial occlusion given intra-arterial doses of 6.5 to 12 mg of compound, SakSTAR.M38 and SakSTAR.M89 induced significantly less neutralizing antibodies and staphylokinase-specific IgG than wild-type SakSTAR[43]. A systematic reversal to the wild type residue of one or more of the substituted amino acids of SakSTAR.M38 (ref. 45, 46) revealed that approximately 40% of the staphylokinase-specific IgG elicited in patients by treatment with wild type SakSTAR required lysine in position 74 of the molecule for binding[45]. The variant SakSTAR (K_{74}), with a single substitution of lysine 74 for alanine had an intact specific activity and thrombolytic potency, but induced significantly less antibody formation in patients than wild type SakSTAR[46]. Thus, these variants provide proof of concept that reduction of the immunogenicity and immunoreactivity in man of recombinant staphylokinase is feasible with protein engineering.

Conclusions

Staphylokinase is a 136 amino acid protein with profibrinolytic properties that forms a 1:1 stoichiometric complex with plasminogen which, following conversion of plasminogen to plasmin and processing of the staphylokinase molecule by hydrolysis of the lysine10-lysine 11 peptide bond, is able to activate other plasminogen molecules to plasmin. The plasmin.staphylokinase complex, unlike the plasmin.streptokinase complex, is rapidly inhibited by α_2-antiplasmin. In a plasma milieu, staphylokinase is able to dissolve fibrin clots without associated plasminogen activation and fibrinogen degradation in the plasma. This fibrin-selectivity of staphylokinase is the result of reduced inhibition by α_2-antiplasmin of plasmin.staphylokinase complex bound to fibrin, recycling of staphylokinase from the plasmin.staphylokinase complex following inhibition by α_2-antiplasmin, and prevention by α_2-antiplasmin of processing of staphylokinase and of conversion of plasminogen.staphylokinase to plasmin.staphylokinase in plasma.

Fibrin-selective thrombolysis with recombinant staphylokinase has been obtained with high frequency in patients with acute myocardial infarction and with peripheral arterial occlusion, but this induced neutralizing antibodies against staphylokinase after a lag period of 1 to 2 weeks in most patients. Reduction of the immunogenicity of staphylokinase, however, appears to be feasible. Definition of the therapeutic benefit of recombinant staphylokinase and non-immunogenic variants

thereof will require more detailed dose-finding studies, followed by large comparative clinical trials against currently used thrombolytic agents. The results of ongoing studies suggest that potent poorly immunogenic variants of staphylokinase will become available for highly fibrin-selective thrombolytic therapy of thromboembolic disease towards the year 2000.

Acknowledgments

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ERRATA

The figures have been interchanged.

Fig. 1: "Coronary artery showing atherosclerotic plaques and a thrombus".

This figure goes with the introduction in the heading and should not have been numbered.

Fig. 2: "Schematic representation of the fibrinolytic system...".

This is figure 1 referred to in the text.

Fig.3: "Molecular interactions determining the fibrin-selectivity of ..."

This is figure 2 referred to in the text.

Fig. 3: Has been deleted, should have been

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1                                                          15
Ser Ser Ser Phe Asp Lys Gly Lys Tyr Lys Lys Gly Asp Asp Ala
16                                                         30
Ser Tyr Phe Glu Pro Thr Gly Pro Tyr Leu Met Val Asn Val Thr
31                                                         45
Gly Val Asp Ser Lys Gly Asn Glu Leu Leu Ser Pro His Tyr Val
46                                                         60
Glu Phe Pro Ile Lys Pro Gly Thr Thr Leu Thr Lys Glu Lys Ile
61                                                         75
Glu Tyr Tyr Val Glu Trp Ala Leu Asp Ala Thr Ala Tyr Lys Glu
76                                                         90
Phe Arg Val Val Glu Leu Asp Pro Ser Ala Lys Ile Glu Val Thr
91                                                        105
Tyr Tyr Asp Lys Asn Lys Lys Lys Glu Glu Thr Lys Ser Phe Pro
106                                                       120
Ile Thr Glu Lys Gly Phe Val Val Pro Asp Leu Ser Glu His Ile
121                                                       135
Lys Asn Pro Gly Phe Asn Leu Ile Thr Lys Val Val Ile Glu Lys
136
Lys
```

Fig. 4: OK