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Molecular Mechanisms of Fibrinolysis and Their Application to Fibrin-Specific Thrombolytic Therapy

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The fibrinolytic system comprises a proenzyme, plasminogen, which can be converted to the active enzyme, plasmin, which degrades fibrin. Plasminogen activation is mediated by plasminogen activators, which are classified as either tissue-type plasminogen activators (t-PA) or urokinase-type plasminogen activators (u-PA). Inhibition of the fibrinolytic system may occur at the level of the activators or at the level of generated plasmin.

Plasmin has a low substrate specificity, and when circulating freely in the blood it degrades several proteins including fibrinogen, factor V, and factor VIII. Plasma does, however, contain a fast-acting plasmin inhibitor, α_2-antiplasmin, which inhibits free plasmin extremely rapidly but which reacts much slower with plasmin bound to fibrin. A "systemic fibrinolytic state" may, however, occur by extensive activation of plasminogen and depletion of α_2-antiplasmin. Clot-specific thrombolysis therefore requires plasminogen activation restricted to the vicinity of the fibrin.

Two physiological plasminogen activators, t-PA and single-chain u-PA (scu-PA) induce clot-specific thrombolysis, via entirely different mechanisms, however. t-PA is relatively inactive in the absence of fibrin, but fibrin strikingly enhances the activation rate of plasminogen by t-PA. This is explained by an increased affinity of fibrin-bound t-PA for plasminogen and not by alteration of the catalytic rate constant of the enzyme. The high affinity of t-PA for plasminogen in the presence of fibrin thus allows efficient activation on the fibrin clot, while no significant plasminogen activation by t-PA occurs in plasma. scu-PA has a high affinity for plasminogen (K_m = 0.3 μM) but a low catalytic rate constant (k_{cat} = 0.02 sec^{-1}). However, scu-PA does not activate plasminogen in plasma in the absence of a fibrin clot, owing to the presence of (a) competitive inhibitor(s). Fibrin-specific thrombolysis appears to be due to the fact that fibrin reverses the competitive inhibition.

The thrombolytic efficacy and fibrin specificity of natural and recombinant t-PA has been demonstrated in animal models of pulmonary embolism, venous thrombosis, and coronary artery thrombosis. In all these studies intravenous

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infusion of t-PA at sufficiently high rates caused efficient thrombolysis in the absence of systemic fibrinolytic activation.

The efficacy and relative fibrinogen-sparing effect of t-PA was recently confirmed in three multicenter clinical trials in patients with acute myocardial infarction. Intravenous infusion of 0.5–1 mg of t-PA per kg body weight over 1–3 hr resulted in coronary reperfusion in approximately 70% of patients. It raised the plasma level about 1,000-fold but was associated with an average decrease of the plasma fibrinogen level by 30%.

Specific thrombolysis by scu-PA has also been demonstrated in animal models of pulmonary embolism, venous thrombosis, and coronary artery thrombosis. Again, intravenous infusion of scu-PA at sufficiently high rates caused thrombolysis in the absence of systemic fibrinolytic activation. We have treated six patients with acute myocardial infarction with scu-PA and have obtained coronary reperfusion during intravenous infusion of 40 mg scu-PA over 60 min in four of the patients and during subsequent intracoronary infusion in one additional patient. A decrease of fibrinogen to 25% of the preinfusion value was observed in one patient.

Key words: fibrinolysis, thrombolysis, fibrin-specificity, plasminogen activators

Mammalian blood contains an enzymatic system capable of dissolving blood clots, which is called the fibrinolytic enzyme system. This system comprises a proenzyme, plasminogen, which can be converted to the active enzyme plasmin, that will degrade fibrin. Activation of plasminogen can be induced by several enzymes, called plasminogen activators. Inhibition of the fibrinolytic system may occur at the level of the activators or at the level of plasmin.

MAIN COMPONENTS OF THE FIBRINOLYTIC SYSTEM

Plasminogen

Human plasminogen is a single-chain glycoprotein with a molecular weight of about 92,000 that contains about 2% carbohydrate. The plasminogen molecule consists of 790 amino acids; it contains 24 disulfide bridges and five homologous triple-loop structures or "kringles" [1]. Native plasminogen has NH_2-terminal glutamic acid ("Glu-plasminogen") but is easily converted by limited plasmic digestion to modified forms with NH_2-terminal lysine, valine, or methionine, commonly designated "Lys-plasminogen." This conversion occurs by hydrolysis of the Arg 67-Met 68, Lys 76-Lys 77, or Lys 77-Val 78 peptide bonds. The concentration of plasminogen in plasma is about 2 μM.

Plasminogen is converted to plasmin by cleavage of a single Arg-Val bond [2] corresponding to the Arg 560-Val 561 bond. The two-chain plasmin molecule is composed of a heavy chain or A-chain, originating from the NH_2-terminal part of plasminogen, and a light chain or B-chain, constituting the COOH-terminal part. The B-chain was found to contain an active site similar to that of trypsin, composed of His 602, Asp 645, and Ser 740 [1].

The plasminogen molecule contains structures, called lysine-binding sites, that interact specifically with certain amino acids such as lysine, 6-aminohexanoic acid, and trans-4-aminomethylcyclohexane-1-carboxylic acid (tranexamic acid) [3]. Plasminogen contains one binding site with high affinity for 6-aminohexanoic acid (K_d = 9 μM) and about four with low affinity (K_d = 5 mM) [4]. These lysine-binding sites are located in the plasmin A-chain [5]. The high affinity lysine-binding site has been shown to be comprised within the first kringle structure of plasminogen [6].

Plasminogen can specifically bind to fibrin through its lysine-binding sites; 6-aminohexanoic acid abolishes the adsorption of plasminogen to fibrin. The lysine-binding sites in plasminogen mediate its interaction with fibrin and with α_2-antiplasmin [7] and histidine-rich glycoprotein [8]. On the basis of these interactions it was suggested that the lysine-binding sites play a crucial role in the regulation of fibrinolysis [9,10].

Plasminogen Activators and Inhibitors of Plasminogen Activators

Plasminogen activation may occur via different pathways, but all mechanisms of plasminogen activation studied so far occur by hydrolysis of the Arg 560-Val 561 peptide bond in plasminogen, yielding the two-chain plasmin molecule. Some of these activators are counterbalanced by inhibitors.

"Intrinsic" activation. In the so-called intrinsic or humoral pathway of plasminogen activation, all the components involved (factor XII, prekallikrein, high molecular weight kininogen, etc) are present in precursor form in the blood. Both factor XII-dependent and -independent activator activities have been reported [11]. The biological role, however, is not well established.

Urokinase and single-chain urokinase-type plasminogen activator (scu-PA). Urokinase is a trypsin-like serine protease composed of two polypeptide chains (M_r 20,000 and 34,000) connected by a single disulfide bridge. It is isolated from human urine or cultured human embryonic kidney cells. Urokinase activates plasminogen directly to plasmin. The complete primary structure of high molecular weight urokinase has been elucidated [12]; the light chain contains 157 amino acids, and the heavy chain contains 253. Plasmin can hydrolyse the Lys 135-Lys 136 peptide bond to yield low M_r urokinase with M_r 33,000.

Evidence that urokinase is secreted in an "inactive" form (scu-PA), which can be activated by plasmin, was already provided in 1973 [13] and in 1977 [14], but the mechanism of activation remained unknown. scu-PA has been purified from urine [15], plasma [16], conditioned cell culture media [17–20], and transformed bacteria [21]. We have recently developed a simple purification method for scu-PA from both conditioned cell culture media and from urine, based on chromatography on zinc-chelate-Sepharose, SP-Sephadex, and Sephadex G-100 [22]. scu-PA is a single-chain glycoprotein containing 411 amino acids and 24 cysteine residues [12,21]. Upon limited plasmin hydrolysis of the Lys 158-Ile 159 peptide bond, the molecule is converted to urokinase, a two-chain molecule linked by one disulfide bridge. The catalytic center is located in the carboxy-terminal chain and is composed of Asp 255, His 204, and Ser 356. Husain et al have claimed that scu-PA has a high affinity for fibrin [23], but this was not confirmed [24,25]. scu-PA is virtually inactive towards low M_r substrates, which are very reactive with urokinase [15,25]; it does not react with the fast-acting inhibitor of t-PA and urokinase.

Streptokinase. Streptokinase is a nonenzyme protein with M_r 47,000, produced by Lancefield group C strains of beta-hemolytic streptococci, which activates the fibrinolytic system indirectly. Streptokinase initially forms a 1:1 stoichiometric complex with plasminogen, which then undergoes a transition, allowing formation of a complex that exposes an active site in the modified plasminogen moiety. This complex then enzymatically converts plasminogen to plasmin.

Streptokinase is at present the most widely used thrombolytic agent, but its optimal dose regimen and its exact place in the treatment of thromboembolic disease are still debated [26].

Tissue-type plasminogen activator (t-PA). The first satisfactory purification procedure for human t-PA has been developed with human uterine tissues [27]. t-PA has been purified from the culture fluid of a stable human melanoma cell line (RPMI-7272) [28]. Sufficient amounts were obtained to study its biochemical and biological properties. The gene of human t-PA has been cloned and expressed [29]. Human t-PA, obtained by expression of recombinant DNA coding for its entire sequence in eukaryotic cells, was shown to be indistinguishable from the natural activator isolated from human melanoma cell cultures, with respect to biochemical properties, turnover in vivo, and specific thrombolytic effect [30].

Native t-PA is a serine protease with a molecular weight of about 70,000, composed of one polypeptide chain containing 527 amino acids, 35 cysteine residues, and four potential N-glycosylation sites (Asn 118, 186, 218, and 448) [29]. Upon limited plasmic action the molecule is converted to a two-chain activator linked by one disulfide bond [28,31]. This occurs by cleavage of the Arg 275-Ile 276 peptide bond yielding a heavy chain (M_r 31,000) derived from the NH_2-terminal part of the molecule and a light chain (M_r 28,000) that comprises the COOH-terminal region. The heavy chain of t-PA contains two kringle regions of 82 amino acids each (residues 92-173 and 180-261), which share a high degree of homology with the five kringles of plasminogen and with similar kringles in prothrombin and urokinase.

The catalytic site located in the light chain of t-PA is composed of His 322, Asp 371, and Ser 478. The amino acid sequences surrounding these residues are highly homologous to corresponding parts of other serine proteases [29]. Comparison of the primary structures of high M_r urokinase and t-PA has revealed a high degree of homology between the two proteins, except that t-PA contains a 43-residue-long amino-terminal region, which has no counterpart in urokinase; this segment was shown to be homologous with the finger-domains responsible for the fibrin affinity of fibronectin. Limited proteolysis of this region has been claimed to lead to a loss of the fibrin affinity of the enzyme [32].

The one-chain and two-chain forms of t-PA have virtually the same fibrinolytic and plasminogen-activating properties [31,33]. The one-chain activator is quickly converted to a two-chain form on the fibrin surface, and therefore it was suggested that physiological fibrinolysis induced by native one-chain plasminogen activator nevertheless occurs, mainly via a two-chain derivative [33].

t-PA is a poor enzyme in the absence of fibrin, but fibrin strikingly enhances the activation rate of plasminogen [34]. This has been explained by an increased affinity of fibrin-bound t-PA for plasminogen [35], which allows efficient fibrin-specific plasminogen activation.

α_2-Antiplasmin

For a long time it was accepted that there were essentially two functionally important plasmin inhibitors in plasma: an immediately reacting one and a slow reacting one identical with α_2-macroglobulin and α_1-antitrypsin, respectively [36]. Later, however, a new plasmin inhibitor that occurs in human plasma has been described, α_2-antiplasmin [37–40]. Upon activation of plasminogen in plasma, the formed plasmin is first preferentially bound to this inhibitor; only upon complete activation of plasminogen (concentration about 2 μM), resulting in saturation of this plasmin inhibitor (concentration about 1 μM), is the excess plasmin neutralized by α_2-macroglobulin.

α_2-Antiplasmin is a single-chain glycoprotein with a M_r of 70,000 that contains about 14% carbohydrate. The molecule consists of 452 amino acids and contains two disulfide bridges [41]. α_2-Antiplasmin belongs to the protein superfamily named serpins (serine protease inhibitors [41]).

In purified systems [38,42] and in plasma [37,39], α_2-antiplasmin forms a 1:1 stoichiometric complex with plasmin, which is devoid of protease or esterase activity. α_2-Antiplasmin, like many other plasma proteinase inhibitors, has a broad in vitro inhibitory spectrum, but its physiological role as an inhibitor of proteases other than plasmin seems negligible.

The kinetics of the inhibition of human plasmin by α_2-antiplasmin have been extensively studied [43,44]. The disappearance of plasmin activity after addition of excess α_2-antiplasmin does not follow first-order kinetics. Most of the plasmin is very rapidly inactivated, but the process only slowly proceeds towards completion. This time course of the reaction is compatible with a kinetic model composed of two successive reactions: a very fast reversible second-order reaction, followed by a slower irreversible first-order reaction.

The second-order rate constant of the first reaction is $2\text{–}4 \times 10^7 \cdot M^{-1} \cdot sec^{-1}$ [45]. This is among the fastest protein-protein reactions so far described. Such rate constants approach the theoretical values for a diffusion-controlled process. The dissociation constant of the reversible complex is 2×10^{-10} M. The rate constant of the first-order transition is $4.2 \times 10^{-3} \cdot sec^{-1}$ [45].

Plasmin molecules that have a synthetic substrate bound to their active site or 6-aminohexanoic acid bound to their lysine-binding site(s) [45,46] do not react or react only very slowly with α_2-antiplasmin. The first step of the process is thus clearly dependent on the presence of a free lysine-binding site and an active site in the plasmin molecule.

MECHANISM OF CLOT-SPECIFIC THROMBOLYSIS

Plasmin, the proteolytic enzyme of the fibrinolytic system, is a serine protease with a relatively low substrate specificity. When plasmin circulates freely in the blood, it will degrade a number of plasma proteins, including fibrinogen and the blood coagulation factors V and VIII. Plasma does, however, contain a fast-acting plasmin inhibitor, α_2-antiplasmin, which reacts extremely rapidly with plasmin [44]. Thus, small amounts of plasmin formed in the blood will be inhibited with a t1/2 of 0.1 sec. The rapidity of this reaction is, however, dependent on the availability of lysine-binding sites in the plasmin molecule and on the availability of a free active site in the enzyme. Plasmin generated on the fibrin surface has both its lysine-binding sites and its active site occupied and is, therefore, only slowly inactivated by α_2-antiplasmin. Thus, plasmin at the fibrin surface is out of the reach of α_2-antiplasmin and is able to lyse fibrin locally. Plasmin generated in the circulating blood, however, will rapidly be neutralized by α_2-antiplasmin and thereby be lost for thrombolysis. Extensive systemic activation of the fibrinolytic system will cause depletion of α_2-antiplasmin, resulting in an excess of circulating free plasmin activity and the development of a systemic fibrinolytic state [10]. The clot specificity that t-PA and scu-PA exert in a plasma environment appears to occur via different mechanisms.

t-PA

As noted above, t-PA is relatively inactive in the absence of fibrin, but fibrin strikingly enhances the activation rate of plasminogen by t-PA. This is explained by

an increased affinity of fibrin-bound t-PA for plasminogen and not by alteration of the catalytic rate constant of the enzyme [35]. The kinetic data of Hoylaerts et al [35] support a mechanism in which t-PA and plasminogen adsorb to a fibrin clot in a sequential and ordered way; ie, t-PA binds first to fibrin and plasminogen then binds to the bimolecular complex, which yields a cyclic ternary complex. Fibrin essentially increases the local plasminogen concentration by creating an additional interaction between t-PA and plasminogen. The high affinity of t-PA for plasminogen in the presence of fibrin thus allows efficient activation on the fibrin clot; no significant plasminogen activation by t-PA occurs in plasma.

scu-PA

The following hypothetical mechanism for the clot specificity of scu-PA is compatible with our present findings [21,25]. scu-PA has a much higher affinity for plasminogen than does urokinase, but it has a lower catalytic rate constant. However, scu-PA does not activate plasminogen in plasma in the absence of a fibrin clot, owing to competitive inhibition. Fibrin neutralizes the competitive inhibition, but this does not seem to occur via specific binding of scu-PA to fibrin. The detailed molecular interactions that regulate the fibrin-specific activation of the fibrinolytic system by scu-PA remain to be elucidated. Other authors have, however, claimed that scu-PA is inactive towards plasminogen and therefore constitutes a real proenzyme [47].

THROMBOLYTIC PROPERTIES IN ANIMAL MODELS OF THROMBOSIS

t-PA

The thrombolytic properties of t-PA obtained either from conditioned cell culture media or by recombinant DNA methods have been evaluated in animal models of pulmonary embolism [48,49], venous thrombosis [30,50–52], and coronary artery thrombosis [53–56]. The thrombolytic effects of t-PA and urokinase were compared in rabbits with experimental pulmonary emboli [48]. t-PA caused thrombolysis at lower doses than did urokinase (on a molar basis). Thrombolysis with t-PA was achieved without extensive plasminogen activation in the circulating blood and without fibrinogen breakdown. Carlin et al [49] induced lysis of intravascular fibrin deposits in the lungs of rats following infusion of human t-PA. In dogs with experimental thrombosis of the femoral vein, intravenous infusion of t-PA caused thrombolysis without associated fibrinogen breakdown [50]. In a preliminary study, Sampol et al [51] reported successful recanalization of thrombosed dog femoral veins with porcine t-PA. In rabbits with experimental jugular vein thrombosis, the extent of thrombolysis of t-PA was determined mainly by the dose of t-PA (1 mg over 4 hr, yielding 80% lysis) and its delivery in the vicinity of the thrombus (local infusion 5 times more efficient), and much less by either the age of the thrombus (up to 7 days) or the molecular form of the activator [52]. Equivalent thrombolytic potencies of t-PA purified from conditioned cell culture media or obtained by recombinant DNA technology were established in the rabbit jugular vein thrombosis model [30]. In dogs with a 1–2-hr-old left anterior descending (LAD) coronary artery thrombus induced with a copper coil, thrombolysis was obtained by intravenous infusion of 5–10 μg/kg/min of human t-PA obtained from melanoma cell cultures [53] or by recombinant DNA technology [54]. In addition to inducing clot lysis, infusion of t-PA also restored intermediary metabolism and nutritional blood flow, without causing sys-

temic fibrinolytic activation. Gold et al [55] found a linear dose-dependent correlation between the rate of infusion of recombinant t-PA and the time to reperfusion in dogs with a 2-hr-old coronary artery thrombus. Timely reperfusion was associated with substantial salvage of myocardial tissue as judged by vital staining of heart muscle; this was obtained in the absence of systemic fibrinogen breakdown. Flameng et al [56] produced a coronary thrombus in baboons, obtained reperfusion by intravenous administration of 10 μg/kg/min of recombinant t-PA, and found a linear correlation between coronary occlusion time and the infarct size. Agnelli et al [57] used a quantitative bleeding model in rabbits consisting of standard incisions in the ear and quantitation of blood loss to demonstrate that t-PA, in comparison with streptokinase, provokes much less hemorrhage at equivalent thrombolytic doses.

scu-PA

Several groups have compared the thrombolytic effects of scu-PA and urokinase in animal models [58–62]. By intravenous administration of 3,000 IU of scu-PA or urokinase per kg body weight in dogs with an experimental thrombosis of the saphenous vein, Sumi et al [58] obtained complete thrombolysis within 1.5 hr with scu-PA. The lysis time was more than 3 hr in the group treated with urokinase. Gurewich et al [59] studied the thrombolytic effect of scu-PA of human kidney cell origin and of urokinase in rabbits and dogs with intravenously injected radioactive fibrin strands. In rabbits, the mean extent of fibrinolysis after 5 hr was highest following infusion of scu-PA. Infusion or urokinase was accompanied by systemic fibrinogenolysis, while scu-PA did not cause significant fibrinogen degradation. Dogs were found to be about ten times more sensitive to human urokinase than rabbits; otherwise the results in dogs were similar to those in rabbits. The thrombolytic properties of recombinant scu-PA (rec-scu-PA), recombinant urokinase (rec-UK), and natural urinary urokinase (nat-UK) were compared in rabbits with a radiolabeled thrombus in the jugular vein [60]. The thrombolytic agents were infused intravenously over a period of 4 hr. Significant thrombolysis with nat-UK and rec-UK were obtained only with 240,000 IU/kg or more. This was associated with a marked systemic activation of the fibrinolytic system, as evidenced by consumption of plasminogen and α_2-antiplasmin, and fibrinogen breakdown. Infusion of rec-scu-PA induced thrombolysis at a dose of 60,000 IU/kg or more without producing systemic fibrinolysis. The specific thrombolytic activity of t-PA was, however, two- to fourfold higher than that of rec-scu-PA.

In dogs with a 1-hr-old left anterior descending coronary artery thrombus induced with a copper coil, thrombolysis was attempted by intravenous infusion of scu-PA isolated from a transformed human kidney cell line. Coronary reperfusion was obtained within 23 ± 2 min with an infusion rate of 20 μg/kg/min, but not within 30 min at 10 μg/kg/min. There was no systemic activation of the fibrinolytic system. Intravenous urokinase was about equipotent in terms of thrombolysis but was associated with extensive systemic fibrinolytic activation [61]. Flameng et al used open-chest baboons with thrombin-induced occlusive thrombus of the left anterior descending coronary artery [62]. Intravenous fusion of rec-scu-PA at a rate of 20 μg/kg/min produced persistent reperfusion within 21 ± 4 min, which was associated with a significant reduction of infarct size and restoration of microvascular blood flow. Infusion of rec-scu-PA was not associated with systemic activation of the fibrinolytic system, fibrinogen breakdown, or bleeding.

THROMBOLYTIC PROPERTIES IN MAN

t-PA

t-PA was first used to treat patients in 1981, when intravenous administration of human t-PA (7.5 mg over 24 hr) induced complete lysis of a 6-week-old renal and iliofemoral thrombosis in a renal allograft recipient [63]. Thrombolysis was achieved without systemic fibrinolytic activation or fibrinogen breakdown and was not associated with bleeding. In a second patient with thrombi in the iliac vein, vena cava, and right renal vein, intravenous infusion of 5 mg of t-PA over 24 hr resulted in resolution of the thrombi. No side effects were noted, and again this thrombolytic therapy was not associated with consumption of fibrinogen, plasminogen, or α_2-antiplasmin [63]. However, in four patients who had deep vein thrombosis of several days duration over extended segments of the iliac and femoral veins, intravenous infusion of 5–25 mg of t-PA over 24–36 hr did not result in thrombolysis [64]. The first pilot study of t-PA in seven patients with acute myocardial infarction was carried out with the use of purified t-PA obtained from melanoma cell culture fluid [65]. Intravenous or intracoronary administration of t-PA in doses of 20,000–40,000 IU (200–400 μg) per min for 30–60 min (total dose up to 20 mg) induced coronary thrombolysis within 19–50 min in six of seven patients. The results were confirmed angiographically in each case [65]. Circulating fibrinogen, plasminogen, and α_2-antiplasmin were not depleted. In the one patient in whom lysis was not inducible with t-PA, it also was not inducible with streptokinase. This first study with t-PA isolated from conditioned cell culture media was followed by a study with recombinant t-PA in 50 patients with acute myocardial infarction of less than 6 hours duration, which revealed that intravenous infusion of 500 μg recombinant t-PA per kg over 60 min (40 mg total) or of the same dose followed by infusion of 250 μg of t-PA per kg over an additional 60 min (60 mg total) resulted in reperfusion of occluded coronary arteries in 75% of the patients. Reperfusion was obtained without significant systemic fibrinogen breakdown in the majority of the patients but not in all of them [66]. The results of this first study with recombinant t-PA in patients with acute myocardial infarction served as a basis for the design of the TIMI-trial in the United States and of the European Cooperative trials. In the TIMI-trial, recombinant t-PA was infused over 3 hr (500 μg/kg over the first hr and 250 μg/kg during each of the 2 subsequent hrs) and compared to 1.5 million units of streptokinase infused over 1 hr. After an initial open-label phase carried out in 87 patients, this study was performed double blind in 316 patients. The results showed that recombinant t-PA was a more effective agent than streptokinase for the reperfusion of occluded coronary arteries and that reperfusion was obtained with less extensive fibrinogen breakdown [67]. In the first European Cooperative trial, recombinant t-PA was infused at a rate of 0.75 mg/kg over 90 min and compared to 1.5 million units of streptokinase infused over 1 hr. This study, carried out in 129 patients, also showed that recombinant t-PA was more effective for coronary thrombolysis than streptokinase and that it induced less fibrinogen breakdown [68]. In the second European trial, recombinant t-PA was infused at a rate of 0.75 mg/kg over 90 min and was compared to placebo in 129 patients. The patency rate with t-PA was 61% as compared to 21% for the placebo group. Although t-PA infusion resulted in a decrease of fibrinogen to about 50%, no major bleeding complications were observed [69]. The congruent conclusions of all four clinical trials with recombinant t-PA reported to date thus constitute a solid base for the further evaluation of

thrombolysis in the management of acute myocardial infarction. The thrombolytic potential of t-PA now seems to be established, but the influence of coronary reperfusion on cardiac irritability and cardiac hemorrhage and the benefit of coronary thrombolysis for the patient remain to be further investigated.

scu-PA

We have treated six patients with scu-PA obtained from human cell culture media [70] and 17 patients with recombinant scu-PA [71]. Intravenous administration of 40–70 mg scu-PA over 60 min resulted in coronary reperfusion of the infarct-related coronary artery in 75% of the patients but was associated with a decrease of fibrinogen to below 20% of the preinfusion value in about 25% of the patients.

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