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The Plasminogen (Fibrinolytic) System

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Introduction

The plasminogen (fibrinolytic) system (Fig. 1) comprises an inactive proenzyme, plasminogen, that can be converted to the active enzyme, plasmin. Plasmin degrades fibrin and activates matrix metalloproteinases (MMPs) that, in turn, degrade the extracellular matrix (ECM).[1-3] Two physiological plasminogen activators (PAs) have been identified: tissue-type PA (t-PA) and urokinase-type PA (u-PA), which binds to a cellular u-PA receptor (u-PAR). Inhibition of the plasminogen/MMP system occurs at the level of the PA, by specific PA inhibitors (PAIs), at the level of plasmin, primarily by α_2-antiplasmin, or at the level of MMPs, by tissue inhibitors of MMPs (TIMPs).

The dual roles of the plasminogen system are presently well established. The t-PA-mediated pathway is primarily involved in fibrin homeostasis, and the u-PA-mediated pathway is primarily involved in phenomena, such as cell migration and tissue remodeling. Consequently, the terminology "fibrinolytic system" has become inadequate and, therefore, will be replaced by "plasminogen system" in the present review.

In 1980, the state of knowledge concerning the plasminogen system was summarized.[4] At that time, most of the components of the system (except the PAIs) were identified and biochemically characterized (except t-PA), but thrombolytic therapy was still in its infancy. The pathophysiologic role of the plasminogen system was deduced indirectly from correlations between levels of its components and clinical disease states, whereas its role in vascular biology, matrix remodeling, tumor growth and dissemination, wound healing, and infection was largely unknown. The last 20 years have witnessed a rapidly progressing elucidation of the biochemistry, (patho)physiology, and therapeutic applications of the plasminogen system. This development has been catalyzed by the emergence of powerful molecular biological technologies, including recombinant DNA techniques for the expression of heterologous proteins and targeted gene manipulation in vivo for the elucidation of the (patho)physiological role of their translation products.

The aim of the present review is to summarize the main developments in the plasminogen field since the 1980s. This account will be incomplete, since references to much significant work were omitted due to space limitations. To alleviate this shortcoming, reference is made primarily to review articles, in which more details and citations to original work can be found.

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Components of the Plasminogen System

The enzymes of the plasminogen system are all serine proteinases. The active site of serine proteinases consists of a "catalytic triad" composed of the amino acids serine, aspartic acid, and histidine. This active site is located in the carboxyl-terminal region of the molecules (serine proteinase part), while the amino-terminal regions contain one or more structural/functional domains (modules). The plasminogen system inhibitors are members of the serpin (serine proteinase inhibitor) superfamily. At the carboxyl-terminal region, plasminogen system inhibitors have a specific, reactive site peptide bond (Arg-X or Lys-X) that is cleaved by their target enzyme, resulting in the formation of an inactive enzyme–inhibitor complex. Some physicochemical and genetic properties of the main components of the plasminogen system are summarized in Table 1. These properties are described in greater detail elsewhere.[5] The physicochemical properties of the components of the MMP system, a family of zinc-dependent endopeptidases,[2] and the interaction between the MMP and the plasminogen system[3] are described in detail elsewhere.

Plasminogen

Plasminogen consists of 791 amino acids, as determined by cDNA sequencing, although originally, protein sequencing identified 790 amino acids. The molecule is organized into seven structural domains, comprising a "preactivation peptide" (amino acid residues 1-77), five sequential homologous kringle domains (disulfide-bonded triple loop structures of about 80 residues each), and the proteinase domain (residues 562-791). The kringle domains contain lysine binding sites and aminohexyl binding sites that play a crucial role in the specific recognition of fibrin, cell surfaces, and α_2-antiplasmin. Plasminogen is converted to plasmin by cleavage of a single Arg561-Val562 peptide bond. The human plasminogen gene is located on the long arm of chromosome 6 at band q26 or q27. Each of the five kringle domains is encoded by two exons separated by a single intron in the middle of each structure. The gene is closely related to that of apolipoprotein (a).

Table 1. Main physicochemical and genetic properties of plasminogen system components

	Mr (kD)	Carbohydrate content (%)	Number of amino acids	Catalytic triad or Reactive site	Plasma Concentration (mg/l)	Gene length (kb)
Plasminogen	92	2	791 (790)*	-	200	52.5
Plasmin	85	2	± 715	His602, Asp645, Ser740	-	
t-PA	68	7	530 (527)*	His322, Asp371, Ser478	0.005	36.6
u-PA	54	7	411	His204, Asp255, Ser356	0.008	6.4
α_2-antiplasmin	70	13	464 (452)*	Arg364, Met365	70	16
PAI-1	52	ND	379	Arg346, Met347	0.05	12.2
PAI-2	47,60	ND	393	Arg358, Thr359	<0.005	16.5
u-PAR	50,60	Variable	283			

t-PA: tissue-type plasminogen activator; scu-PA: single chain urokinase-type plasminogen activator; pro-urokinase; PAI-1: plasminogen activator inhibitor-1; PAI-2: plasminogen activator inhibitor-2; u-PAR: u-PA receptor; ND: not determined.
**The numbering of amino acid residues is usually based on these initially determined incorrect values.*[5]

Tissue-Type Plasminogen Activator

Tissue-type plasminogen activator or t-PA consists of 530 amino acids, although originally 527 were identified. The t-PA enzyme is composed of several domains with homologies to other proteins: a finger domain comprising residues 4-50, a growth factor domain comprising residues 50-87, two kringles comprising residues 87-176 and 176-262, and the protease domain constituted by residues 276-527, comprising the catalytic triad. Binding of t-PA to fibrin is most likely mediated via the finger and the second kringle domains. The human t-PA gene has been localized to chromosome 8 (bands 8.p.12->q.11.2). The proximal promoter sequences contain typical TATA and CAAT boxes and potential recognition sequences for transcription factors (e.g., AP1, NF1, SP1, and AP2).

Single-Chain Urokinase-Type Plasminogen Activator

Single-chain urokinase-type plasminogen activator (scu-PA) or prourokinase (pro-u-PA) is composed of an epidermal growth factor domain, one kringle domain, and a protease domain containing the catalytic triad. The epidermal growth factor domain is responsible for the binding of scu-PA to its receptor, which is present on the surface of a variety of cells. Scu-PA is converted to two-chain u-PA (tcu-PA) by cleavage of the Lys158–Ile159 peptide bond. The human u-PA gene is located on chromosome 10.

Alpha Antiplasmin

The α_2-antiplasmin molecule [α_2-plasmin inhibitor] was originally isolated as a glycoprotein containing 452 amino acids, but it was later shown that native α_2-antiplasmin contains 464 amino acids. It is unique among serpins due to its carboxyl-terminal extension of 51 amino acid residues, which contains a secondary binding site that reacts with the lysine binding sites of the kringles 1-3 of both plasminogen and plasmin. The amino-terminal Gln14 residue of α_2-antiplasmin (Gln2 in the original numbering system) can crosslink to Aα-chains of fibrin, in a process that requires Ca^{2+} and is catalyzed by activated coagulation factor XIII. The gene for human α_2-antiplasmin is located on chromosome 18, bands p11.1-q11.2.

Plasminogen Activator Inhibitors

The two most important PAIs are PAI-1 and PAI-2. A tight binding to the cell-adhesion protein vitronectin, also denoted as S-protein, stabilizes PAI-1. The PAI-1 gene is located on chromosome 7, bands q21.3-q22. PAI-2 exists in two different forms with comparable kinetic properties, a 47 kDa intracellular nonglycosylated form with pI 5.0 and a 60 kDa secreted glycosylated form. The PAI-2 gene is located on chromosome 18 q21-23.

Urokinase-Type Plasminogen Activator Receptor

The specific cell surface u-PAR is synthesized as a 313 amino acid polypeptide, which is post-translationally processed at the carboxyl-terminus into a protein of 283 amino acids that is anchored to the plasma membrane by a glycosyl phosphatidylinositol (GPI) moiety. The u-PA receptor binds all forms of u-PA, which contain an intact growth factor domain, with high affinity. It is composed of three distantly related homologous structural domains, of which the amino-terminal one binds u-PA.

Phenotype of Mice Deficient in Plasminogen System Components

Targeting of genes via homologous recombination in embryonic stem cells has allowed the generation of deficiencies (and, more recently, mutations, conditional expression, or tissue-specific expression) of specific gene products in transgenic mice. Since homozygous deficiencies of several plasminogen system components, including t-PA and u-PA, have not been observed in humans, it was anticipated that inactivation of these genes would cause embryonic lethality. Mice with single or combined deficiencies of t-PA, u-PA, PAI-1, u-PAR, plasminogen, or α_2-AP, however, survived embryonic development and were apparently normal at birth, while no effects on health and survival were observed in t-PA-, u-PAR-, or PAI-1-deficient mice.[9] Some u-PA-deficient mice developed chronic (nonhealing) ulcerations and rectal prolapse, but survival rates were normal. Plasminogen-deficient and combined t-PA:u-PA-deficient mice,

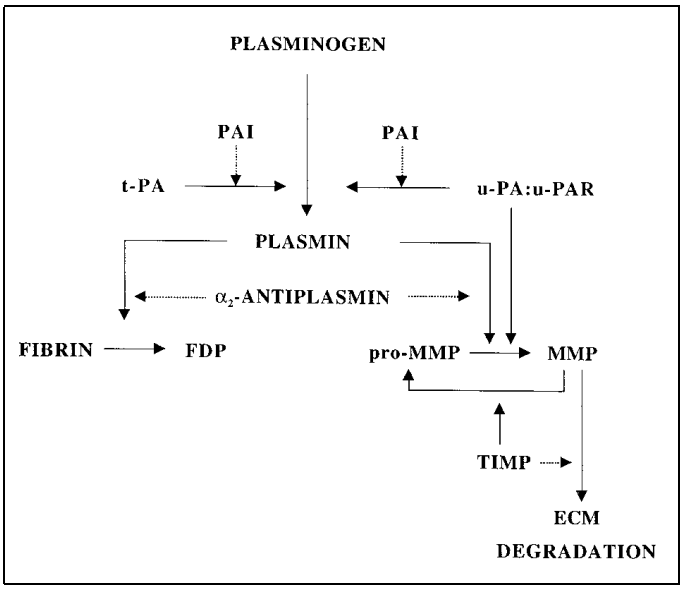

Figure 1. Schematic representation of the plasminogen (fibrinolytic) system. The proenzyme, plasminogen, is converted to the active enzyme plasmin by t-PA or u-PA, which binds to a cellular u-PAR. Plasmin degrades fibrin and can convert latent proMMPs into active MMPs, which, in turn, degrade ECM. ProMMPs may also be activated directly by u-PA or by other MMP. T-PA-mediated plasminogen activation is primarily involved in fibrin homeostasis, while plasmin generation via u-PA, complexed with u-PAR, plays a role in tissue remodeling. Inhibition may occur at the level of the PAs by PAIs (mainly, PAI-1 and, possibly, PAI-2), at the level of plasmin by a_2-antiplasmin and at the level of the MMPs by TIMPs.

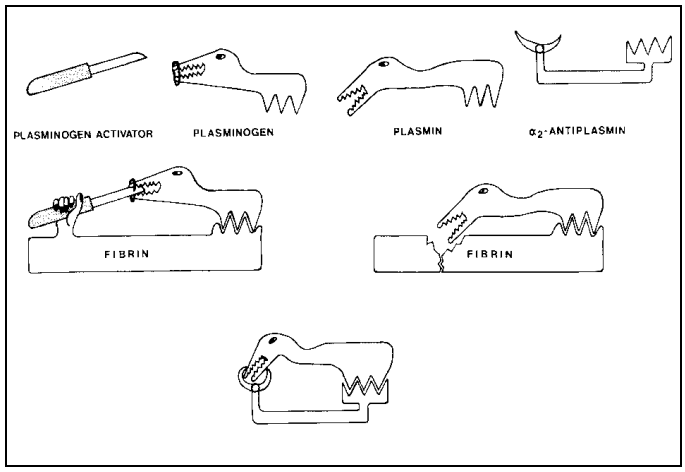

Figure 2. Schematic visualization of the molecular interactions regulating fibrinolysis. Plasminogen is converted to the proteolytic enzyme plasmin by t-PA, but this conversion only occurs efficiently on the fibrin surface, where activator and plasminogen are "assembled." Free plasmin in the blood is very rapidly inactivated by a_2-antiplasmin, but plasmin generated at the fibrin surface is partially protected from inactivation. The lysine binding sites in plasminogen (represented as the "legs" of the animal) are important for the interaction between plasmin(ogen) and fibrin and between plasmin and a_2-antiplasmin (reproduced with permission[6]).

developed similar chronic ulcerations and rectal prolapse. In addition, they suffered a progressive wasting syndrome and displayed a significantly shortened life span due to generalized thrombosis and organ failure.[6] Contrary to patients with low or absent plasma PAI-1 or α_2-AP levels, PAI-1, or α_2-AP-deficient mice did not reveal spontaneous or delayed rebleeding, even after trauma. These gene targeting studies thus confirm the importance of the plasminogen system in maintaining vascular patency and indicate that t-PA and u-PA are the only physiologically significant PAs in vivo that appear to cooperate in fibrin surveillance.

Plasminogen System and Fibrin Homeostasis

Molecular Mechanism of Fibrinolysis

Physiological fibrinolysis appears to be regulated by specific molecular interactions between components of the plasminogen system, which were sufficiently well understood in the early 1980s to allow formulation of the following working hypothesis:[7]

Extrinsic plasminogen activator (later called t-PA) has a weak affinity for plasminogen in the absence of fibrin (K_M = 65 µM) but a much higher affinity in the presence of fibrin (K_M between 0.15 and 1.5 µM). This increased affinity appears to be the result of a "surface assembly" of plasminogen activator and plasminogen on the fibrin surface. In this reaction plasminogen binds to fibrin primarily via specific structures called the "lysine-binding site." Thus one way of regulating fibrinolysis is at the level of plasminogen activation localized at the fibrin surface.

Plasmin is extremely rapidly inactivated by a_2-antiplasmin (k_1» 10^7 M^{-1} sec^{-1}). The half-life of free plasmin in the blood is therefore estimated to be approximately 0.1 seconds. Plasmin with an occupied lysine-binding site is however inactivated 50 times more slowly by a_2-antiplasmin. Reversible blocking of the active site of plasmin with substrate also markedly reduces the rate of inactivation by a_2-antiplasmin. From these findings one can extrapolate that plasmin molecules generated on the fibrin surface, which are bound to fibrin through their lysine-binding sites and involved in fibrin degradation, are protected from rapid inactivation by a_2-antiplasmin. Plasmin released from the fibrin surface would, however, be rapidly inactivated by a_2-antiplasmin. These interactions are schematically visualized in Figure 2.

Although different kinetic constants for the activation of plasminogen by t-PA have subsequently been reported, most studies agree that fibrin stimulates plasminogen activation by t-PA by at least two orders of magnitude. Initial binding of t-PA to fibrin may be governed by the finger domain and by kringle 2, which binds to exposed carboxyl-terminal lysine residues. During fibrin clot lysis, binding of t-PA increases by generation of additional new plasmin-generated carboxyl-terminal lysines.

Control of Plasminogen Activation

Multiple mechanisms are involved in the rapid release of t-PA in human plasma, as discussed in more detail elsewhere.[8] Rapid removal of t-PA from the blood occurs by clearance in the liver via two different recognition systems. Hepatocytes express the low-density lipoprotein receptor-related protein

(LRP) or α_2-macroglobulin receptor, which binds free t-PA and complexes of PAI-1 with t-PA and tcu-PA. Alternatively, endothelial cells express a 170 kDa mannose-dependent receptor. The structures involved in the rapid hepatic clearance of t-PA are localized in the amino-terminal region.

PAI-1 reacts with single-chain t-PA, two-chain t-PA, and tcu-PA, but not with scu-PA. The second-order rate constant for the inhibition of single-chain t-PA by PAI-1 is about 10^7 $M^{-1}s$,[1] while inhibition of two-chain t-PA and tcu-PA is somewhat faster. Positively-charged regions in t-PA (residues 296-304) and in u-PA (residues 179-184) are involved in this rapid interaction. PAI activity is very rapidly cleared from the circulation via the liver.[9]

The synthesis and secretion of t-PA and PAI-1 by endothelial cells is highly regulated,[7] but few agonists stimulate t-PA synthesis without affecting PAI-1 synthesis. Histamine and thrombin bind to specific receptors and activate phospholipase C. Phospholipase C acts on phosphatidyl-inositol bisphosphate to produce diacylglycerol. This activates membrane-bound protein kinase C, which regulates t-PA synthesis. Synthesis and secretion of PAI-1 can be modulated by various agonists.[10] Except for platelets, which contain both functional and inactive PAI-1, PAI-1 is not stored within cells, but is rapidly and constitutively secreted after synthesis.

Most cells bind plasminogen via its lysine binding sites with a high capacity (>10^7 sites per cell), but relatively low affinity (K_d of 1 μM). Gangliosides and membrane proteins with carboxyl-terminal lysine residues, such as α-enolase, also bind plasminogen.[11] Endothelial cells bind t-PA and plasminogen via annexin II and, therefore, may play a role in maintaining blood fluidity. Lp(a) competes with plasminogen for binding and may play a role in the regulation of fibrinolysis at the endothelial cell surface.

Pathophysiology of Fibrinolysis

Impaired Fibrinolysis and Thrombosis

A deficient fibrinolytic response may be caused by impaired release of t-PA from the vessel wall or by an increased rate of neutralization.[12] A causal relationship between deficient synthesis and release of t-PA and thrombosis has, however, not been conclusively established in humans. Transgenic mice which totally lack functional t-PA lyse experimental pulmonary emboli at a markedly reduced rate, but are healthy under basal conditions.

The PAI-1 concentration in plasma is increased in several diseases, including venous thromboembolism, obesity, sepsis, and coronary artery disease. High PAI-1 activity constitutes an independent risk factor for myocardial reinfarction in young subjects within 3 years of the first attack. There is a clear correlation between the circadian variation in the time of onset of myocardial infarction, with the highest incidence at about 8 a.m., and the circadian rhythm of plasma PAI-1 activity, which is also highest early in the morning.

Enhanced Fibrinolysis and Bleeding

Increased levels of t-PA or deficiency of α_2-antiplasmin or PAI-1 may cause a bleeding tendency. Homozygous α_2-antiplasmin deficiency may be associated with a severe hemorrhagic diathesis, whereas heterozygosity causes no or only mild bleeding symptoms.[12] Acquired α_2-antiplasmin deficiency associated with enhanced fibrinolysis has been reported in some conditions, including liver disease, disseminated intravascular coagulation, and acute promyelocytic leukemia.

A life-long hemorrhagic disorder associated with enhanced fibrinolysis, due to increased levels of circulating PA, has been described in a few patients. Alternatively, excessive fibrinolysis due to decreased PAI-1 levels has been reported in a few cases and was apparently associated with bleeding complications. A complete deficiency of PAI-1 has been reported in a 9-year-old girl with episodes of major hemorrhage in response to trauma or surgery.

Thrombolytic Therapy

Major Developments Since 1980

Acute myocardial infarction and ischemic stroke are the first and third causes of death and disability in Western societies. Thrombolytic therapy of these diseases, consisting of the pharmacological dissolution of the blood clot by intravenous infusion of PAs that activate the plasminogen system, is based on the premise that their proximal cause is thrombosis, triggered by the 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. Thrombolytic therapy has been attempted in patients with acute myocardial infarction since the late 1950s, but no significant progress toward its routine clinical use was made until 1980.[13]

The modern era of thrombolytic therapy started around 1980 with the demonstration, by De Wood et al, that myocardial infarction in its early stage was invariably associated with thrombotic coronary artery occlusion. It was also associated with the demonstration by Rentrop et al, following initial work by Chazov et al, that infusion of streptokinase within the infarct-related coronary artery early after symptom onset induced rapid recanalization. The efficacy of intracoronary streptokinase was quickly confirmed and evidence accumulated, both from experimental animal and clinical studies, that timely reopening of a coronary artery led to improved myocardial function. It soon became apparent that widespread application of coronary thrombolysis would depend on the development of simple therapeutic strategies without coronary catheterization.[14] Randomized clinical trials with short-term intravenous streptokinase, initiated by Schröder et al, demonstrated moderate but significant, potency for coronary artery recanalization and culminated, in 1986 in the GISSI trial, which demonstrated a significant overall reduction in mortality with intravenous streptokinase.[13]

In a parallel development in the early 1980s, elucidation of biochemical mechanisms that regulate physiological fibrinoly-

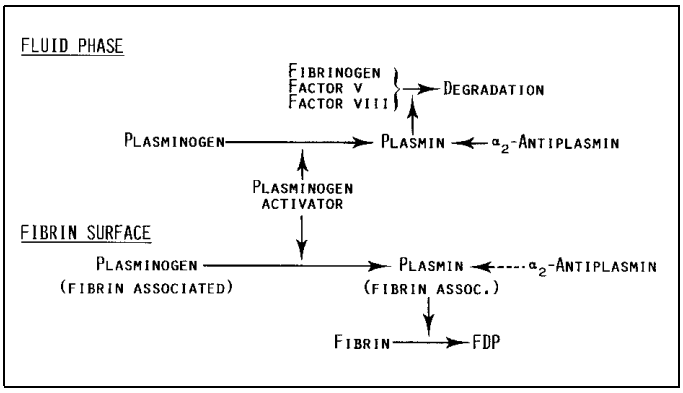

Figure 3. Molecular interactions determining the fibrin specificity of PAs. Nonfibrin-specific PAs (streptokinase, 2-chain urokinase, APSAC, or anistreplase) activate both plasminogen in the fluid phase and fibrin-associated plasminogen. Fibrin-specific PAs (t-PA, scu-PA, and staphylokinase) preferentially activate fibrin-associated plasminogen.

sis provided the conceptual framework for fibrin-selective thrombolysis, schematically represented in Figure 3, which fueled the hope that more specific and efficacious thrombolytic agents could be developed:[7]

The presently available thrombolytic agents streptokinase and urokinase have no specific affinity for fibrin and therefore activate circulating and fibrin-bound plasminogen relatively indiscriminately. Consequently, plasmin formed in circulating blood will initially be neutralized very rapidly by a_2-antiplasmin and be lost for thrombolysis. Once the inhibitor becomes exhausted, residual plasmin will degrade several plasma proteins (fibrinogen, factor V, factor VIII, etc.) and cause a serious bleeding tendency. This may explain why treatment with streptokinase or urokinase has only a limited efficiency and is associated with serious, sometimes life-threatening side effects. From this reasoning it appears that specific thrombolysis will be possible only if the activation process of plasminogen can be localized at and confined to the fibrin surface. According to the present concepts, this can only be adequately achieved with the use of an activator that, like the physiological activator, adsorbs to the fibrin surface and becomes active in loco.

With the development of t-PA for thrombolytic therapy, this hypothesis could be subjected to testing. Initially, two coronary patency studies (TIMI-1 and ECSG-1) supported the higher efficacy of fibrin-selective recombinant t-PA (rt-PA) over non-fibrin-selective streptokinase. Similar results were obtained in several mechanistic trials of the TIMI organization led by Braunwald, the ECSG organization chaired by Verstraete, and the TAMI group of Topol and Califf, which validated and extended the hypothesis of fibrin-selective thrombolytic therapy for acute myocardial infarction.[15] Two subsequent megatrials (GISSI-2 and ISIS-3), however, could not confirm that this translated into a mortality benefit. Unfortunately, these latter two studies omitted the required conjunctive use of heparin anticoagulation, as established in several mechanistic studies in experimental animals and patients[16] and confirmed by meta-analysis results obtained in over 100,000 patients.[17] Finally, the Global Utilization of Streptokinase and rt-PA for Occluded coronary arteries (GUSTO) trial[18] and its angiographic sub-

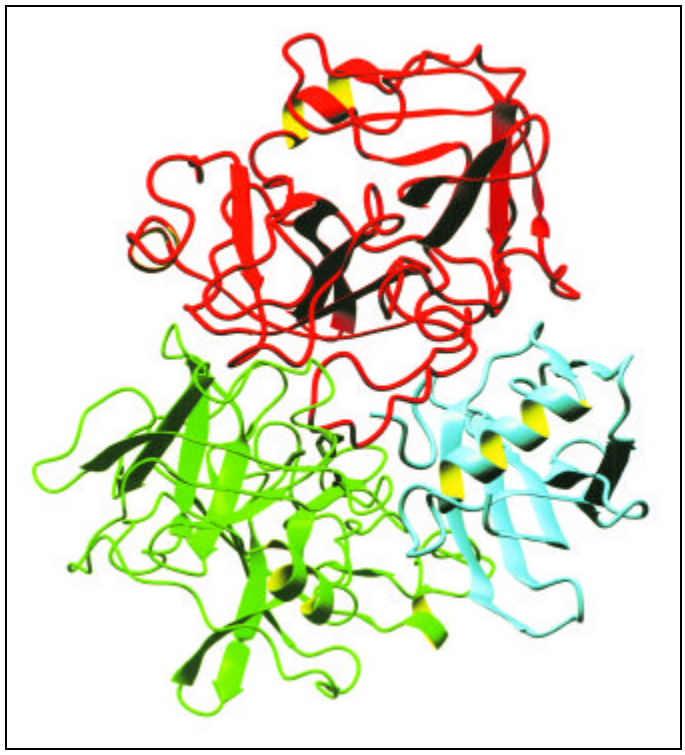

Figure 4. Molecular model of the ternary complex between plasmin (microplasmin, μPli, green), staphylokinase (Sak, blue), and plasminogen (microplasminogen, μPli, red). The molecules are depicted in arrowed ribbon representation, and the side chains of the catalytic triad residues in μPli are represented in dark green sticks. The μPlg substrate molecule is docked in the active site cleft and covers 1140-Å^2 and 1190-Å^2 contact surface with Sak and μPli, respectively. Presented at the XIV International Congress on Fibrinolysis and Thrombolysis, Ljublana, Slovenia, June 22-26, 1998.[30,31]

study[19] revisited the "open artery hypothesis" and conclusively demonstrated that brisk (TIMI 3 flow), early, and persistent coronary artery recanalization is the primary determinant of clinical benefit.[20] A recent meta-analysis of all megatrials reported a different conclusion[21] but made the fundamental mistake of pooling the rt-PA and the rt-PA plus streptokinase groups of GUSTO. These constituted fibrin-selective and non-fibrin-selective thrombolytic strategies with different mortality reductions. A follow-up analysis of the GUSTO results revealed that the difference in mortality persisted after 1 year[22] and that patients who were at higher risk derived a greater absolute benefit from treatment with rt-PA in combination with intravenous heparin than with streptokinase.[23] The beneficial effect of fibrin selectivity, with respect to bleeding, is less convincing. Although stroke-free survival was significantly higher with alteplase than with streptokinase, the rate of intracranial hemorrhage was also slightly, but significantly, higher. In aggregate, however, fibrin selectivity is a desirable property of thrombolytic agents, as discussed in more detail elsewhere.[13]

Table 2. Current Indications/Contraindications and Currently Used Regimens for Thrombolytic Therapy in Acute Myocardial Infarction

A. Indications and contraindications

Current indications

Patients with chest pain consistent with the diagnosis of acute myocardial infarction and at least 0.1 mm of ST-segment elevation in at least two contiguous ECG leads, in whom treatment can be initiated within 12 h of pain onset, provided there are no contraindications to thrombolytic therapy.

Expanded indications

Advanced age, controllable hypertension, a history of nontraumatic cardiopulmonary resuscitation, and left or right bundle branch block are no longer contraindications in patients with significant evidence for evolving myocardial infarction.

Contraindications

History of a serious bleeding tendency; recent acute internal hemorrhages; major surgery, trauma, or delivery within 10 days; traumatic cardiopulmonary resuscitation; vascular puncture in a noncompressible site; and uncontrolled hypertension.

Previous use of streptokinase or anistreplase is a contraindication for their repeated administration for at least 1 year because of their immunogenicity.

B. Currently used regimens

Streptokinase and aspirin

Streptokinase (SK) 1.5 million U IV over 30 to 60 minutes, combined with acetylsalicylic acid (ASA) 160 to 325 mg daily started as soon as possible and continued indefinitely. The safety and efficacy of this regimen in terms of mortality reduction was established in ISIS-2, GISSI-2, and ISIS-3. It is less efficient for mortality reduction in patients treated within the first 6 hours than accelerated alteplase and intravenous heparin, as demonstrated by GUSTO.

Alteplase and intravenous heparin

Alteplase rt-PA 100 mg IV over 90 minutes (15 mg bolus, 0.75 mg/kg not exceeding 50 mg over 30 minutes, and 0.5 mg/kg not exceeding 35 mg over the next 1 hour) combined with 160 mg to 325 mg ASA and immediate intravenous heparin (5,000 U bolus and 1,000 U/hour, preferably monitored with activated partial thromboplastin time). In GUSTO, the accelerated alteplase regimen was associated with a statistically significantly lower mortality than SK (6.3% versus 7.3%, p=0.001) but with a slightly higher incidence (0.1%) of survival with disabling stroke.

Selection of regimen

The GUSTO trial has demonstrated a significant overall survival benefit of accelerated alteplase, given with intravenous heparin over previous regimens, particularly SK with subcutaneous or with intravenous heparin (14% mortality reduction with 95% confidence intervals of 6% to 21%) and a consistent pattern of fewer complications, including allergic reactions, clinical indicators of left ventricular dysfunction, and arrhythmias. The survival benefit is largest in patients <75-years-old, with anterior infarction, and <4 hours from onset of symptoms. No subgroups were identified in which alteplase was significantly worse than SK, but, possibly because of a lack of statistical power, no statistically significant benefit of alteplase compared with SK was documented in patients >75-years-old, in patients with small inferior infarcts, and in patients presenting >4 hours after the onset of symptoms. Therefore, if cost considerations become a limiting factor, alteplase should be reserved primarily for the former subgroups and SK for the latter.

Modified from.[24]

*Currently Used Thrombolytic Agents**

Thrombolytic agents that are either approved or under clinical investigation in patients with acute myocardial infarction include streptokinase, rt-PA or alteplase, rt-PA derivatives (such as reteplase, lanoteplase, and TNK–rt-PA), anisoylated plasminogen–streptokinase activator complex (APSAC or anistreplase), tcu-PA or urokinase, recombinant 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 placebo-controlled clinical trials,[24] and it has become routine treatment.

**This section is based on the special report on "reperfusion in acute myocardial infarction" of the international society and federation of cardiology and World Health Organization Task Force on Myocardial reperfusion. [24] It thus largely reflects the consensus view of this task Force.*

Thrombolytic therapy is given to more than 750,000 patients per year worldwide, while at least three times that number could potentially benefit from this treatment.

The current indications and contraindications to thrombolytic therapy in patients with acute myocardial infarction are summarized in Table 2. Two thrombolytic agents, nonfibrin-selective streptokinase and fibrin-selective alteplase (rt-PA) (Fig. 3), are widely used for intravenous administration in patients with acute myocardial infarction. The currently used regimens for coronary thrombolysis are also briefly summarized in Table 2. Anistreplase, urokinase, and reteplase are approved for thrombolytic therapy in some countries, whereas TNK–rt-PA has recently successfully completed a comparative Phase III clinical trial versus rt-PA in approximately 17,000 patients. Reteplase can be administered as a double bolus injection, and TNK–rt-PA can be administered as a single bolus injection in patients with acute myocardial infarction.

Streptokinase is a bacterial protein that, when added to human plasma, forms a complex with plasminogen; this complex activates other plasminogen molecules to plasmin. The streptokinase–plasmin(ogen) complex is insensitive to circulating proteinase inhibitors and activates circulating and fibrin-bound plasminogen relatively indiscriminately, producing the so-called "systemic lytic state," characterized by fibrinogen degradation and α_2-antiplasmin depletion in circulating blood. The standard dose in patients with acute myocardial infarction is 1.5 million U intravenously infused over 60 minutes. Streptokinase causes transient hypotension in many patients and significant allergic reactions in a small percentage of patients. Its administration causes a rapid rise in antistreptokinase antibody titer after about 4 to 7 days, which is sufficient to neutralize (in vitro) a standard dose of streptokinase and make repeated treatment of uncertain efficacy.

The t-PA molecule is a human protein produced by recombinant DNA technology (recombinant t-PA, rt-PA). t-PA is a poor enzyme in the absence of fibrin, which enhances the activation rate of plasminogen by at least 100 times. Activation of the fibrinolytic system thus seems to be triggered by, and largely confined to, fibrin. Thus far, t-PA has been produced in two forms: a single-chain preparation (alteplase) and a double-chain preparation (duteplase). Most studies have, however, used alteplase. The preferred dosage regimen of fibrin-selective alteplase at present consists of a weight-adjusted, accelerated ("front-loaded") regimen over 90 minutes (15-mg bolus, 0.75 mg/kg over 30 minutes [not to exceed 50 mg] and 0.5 mg/kg over 60 minutes [not to exceed 35 mg]). This accelerated regimen has a survival benefit over streptokinase, as demonstrated in the GUSTO trial.[18,22]

Comparative trials of streptokinase and rt-PA (mostly the single-chain alteplase preparation) have shown significant differences in efficacy for early coronary artery recanalization,[25] and this was confirmed in the angiographic substudy of the GUSTO trial.[19] Accelerated alteplase with intravenous heparin produced somewhat over 50% complete recanalization (TIMI grade 3 flow) at 90 minutes (which is the main predictor of clinical benefit), as compared to around 30% with streptokinase and aspirin.

GUSTO (41,021 patients)[18] compared the outcomes of treatment for up to 30 days with one of four different regimens: 1) streptokinase 1.5 million U intravenous over 60 minutes with 12,500 U heparin given subcutaneously every 12 hours, begun 4 hours after the start of the streptokinase infusion; 2) streptokinase 1.5 million U over 60 minutes with concurrent intravenous heparin in a bolus dose of 5,000 U intravenous and 1,000 U/h, with the dose adjusted to raise the activated partial thromboplastin time (aPTT) to between 60 seconds and 85 seconds; 3) weight-adjusted, accelerated ("front-loaded") alteplase with the same intravenous heparin regimen; and 4) a combination of streptokinase (1 million U intravenous over 60 minutes) and weight-adjusted alteplase (1.0 mg/kg intravenous over 60 minutes, not to exceed 90 mg, with 10% given in a bolus dose) with simultaneous intravenous heparin. Intravenous heparin was given for at least 48 hours, and all patients received aspirin early (≥160 mg), followed by 162 to 325 mg/day.

The 30-day mortality and the rate of disabling stroke in the four groups were as follows: 1) 7.2% and 0.5%, 2) 7.4% and 0.5%, 3) 6.3% and 0.6%, and 4) 7.0% and 0.6%. The 30-day mortality was significantly lower in patients receiving accelerated alteplase and intravenous heparin than in patients receiving streptokinase with either subcutaneous or intravenous heparin ($p=0.001$). A combined endpoint of death or disabling stroke was also significantly lower in the accelerated alteplase group than in the streptokinase-only groups (6.9% versus 7.8%, $p=0.006$). This difference was maintained after 1 year.[22]

Toward Improved Thrombolytic Therapy

Currently available thrombolytic agents have several limitations. At best, TIMI 3 flow within 90 minutes is obtained in somewhat over 50% of patients, acute coronary reocclusion occurs in roughly 10% of patients, coronary recanalization requires on average 45 minutes or more, intracerebral bleeding occurs in 0.3% to 0.7%, and the residual mortality is at least 50% of that without thrombolytic treatment. Furthermore, the most commonly used agents, streptokinase and alteplase, are routinely administered by intravenous infusion over 60 minutes to 90 minutes, which in emergency conditions, is less convenient than bolus injection.

Thrombolytic therapy could be improved in several ways: by reducing the duration of ischemia by earlier and accelerated treatment; by using PAs with increased thrombolytic potency and that can be administered by bolus injection to enhance coronary thrombolysis; and by using more specific and potent anticoagulant and antiplatelet agents to accelerate recanalization and prevent reocclusion.[25]

Patients should receive thrombolytic therapy as soon after the onset of symptoms as possible. In GUSTO, the mortality was 4.3% in patients receiving alteplase within 2 hours after the onset of symptoms, 5.5% for those treated 2 hours to 4 hours after the onset, and 8.9% in those treated 4 hours to 6 hours after the onset of symptoms. The mortality of patients treated with streptokinase in the same time intervals was 5.4%, 6.7%, and 9.3%.[18] Because of this and other compelling evidence, early recanalization must remain the main objective of pharmacological coronary thrombolysis. Continued and intensified education of the public, paramedical personnel, and physicians,

together with the development of rapid and efficient triage systems, are essential to achieve these goals.

Variants of rt-PA have been constructed with altered pharmacokinetic or functional properties, including reduced clearance, altered binding to fibrin and stimulation by fibrin, and resistance to plasma protease inhibitors.[26] In the present overview, only those agents that are being developed for clinical use will be briefly discussed. A deletion mutant, consisting of the kringle 2 and protease domains of rt-PA (reteplase), was used for coronary artery thrombolysis by double bolus administration in two large mortality trials in patients with acute myocardial infarction and was demonstrated to be equipotent to streptokinase (INJECT trial) and, subsequently, to alteplase (GUSTO III trial). Another rt-PA deletion mutant, consisting of the protease domain and both kringle 1 and kringle 2 of rt-PA (lanoteplase), given as a single intravenous bolus injection, is being evaluated in a large mortality trial (Intime II) that has recently completed enrollment. A variant of rt-PA, E6010, in which Cys84 in the E-domain is replaced by Ser, has been used successfully in a multicenter efficacy trial in patients with acute myocardial infarction. An rt-PA mutant, TNK-rt-PA, in which Thr103 is substituted by Asn, Asn117 by Gln, and the sequence Lys296-His-Arg-Arg by Ala-Ala-Ala-Ala, was found to have an eight-fold slower clearance and a 200-fold enhanced resistance to PAI-1. This agent has successfully been tested in a large mortality trial (ASSENT II), as a single intravenous bolus.

Taken together, these studies clearly indicate that rt-PA mutants can be produced with significantly reduced plasma clearance, which, in the case of domain deletion mutants, are also associated with a reduced, specific thrombolytic activity, whereby these molecules do not have a significantly improved thrombolytic potency. As already anticipated some years ago from mechanistic and experimental animal studies,[27] these variants, which have the same catalytic machinery as rt-PA, cannot be expected to outperform native rt-PA, unless the present dosing of alteplase would be suboptimal. Consequently, it should not be surprising if comparative clinical trials of these variants with alteplase designed for equivalence would succeed, while trials designed for superiority would fail.

To obtain thrombolytic agents with an increased thrombolytic potency for coronary recanalization, it will probably be necessary to turn to other PAs with higher specific activity and different mechanisms of fibrin selectivity. Staphylokinase could constitute such an alternative, as further discussed below. The t-PA of saliva from the vampire bat Desmodus rotundus (bat-PA) was found to constitute a potent and fibrin-selective thrombolytic agent in experimental animal models. It is presently under early clinical evaluation. Antibody targeting for the treatment or prevention of thrombi has been recently reviewed in detail by Haber.[5] It entails the engineering of bifunctional molecules that contain both a highly specific antigen binding site that concentrates the molecule at the desired target (the thrombus) and an effector site that will initiate thrombolysis.

Aspirin and heparin have a limited impact on the speed of coronary thrombolysis and on the resistance to lysis, and they do not consistently prevent reocclusion. This could have been anticipated on the basis of the unselective inhibition by aspirin of the synthesis of both proaggregatory and antiaggregatory prostaglandins, and of the relative inefficacy of heparin for the inhibition of clot-associated thrombin.

Several more specific approaches to reduction of platelet aggregation, including monoclonal antibodies against the platelet glycoprotein (GP) IIb/IIIa receptor and small synthetic arginine-glycine-aspartic acid (RGD)-containing peptides, are presently being explored.[28] The concomitant administration of potent platelet GP IIb/IIIa antagonists with aspirin, heparin, and thrombolytic therapy has been shown to be safe and feasible, and phase II studies have been sufficiently encouraging to warrant larger clinical trials, such as TIMI 14, which was recently completed.

Another approach consists of the use of selective thrombin inhibitors, including hirudin and its derivatives, or synthetic thrombin inhibitors. Some of these agents have indeed been shown to be more effective than aspirin or heparin, for the acceleration of arterial recanalization and the prevention of reocclusion, but several clinical trials with thrombolytic agents were terminated prematurely because of excess (cerebral) bleeding.[29] Alternatively, specific inhibitors of factor Xa and the factor VIIa/tissue factor pathway are being explored for conjunctive use with thrombolytic agents.

Staphylokinase, a Potent, Uniquely Fibrin-Selective Thrombolytic Agent

Staphylokinase is a single polypeptide chain of 136 amino acids without disulfide bridges that is secreted by certain strains of *Staphylococcus aureus*. Like streptokinase, staphylokinase is not an enzyme, but it forms a 1:1 stoichiometric complex with plasmin(ogen) that activates other plasminogen molecules. The structure of the ternary plasmin–staphylokinase–plasminogen complex (Fig. 4) has recently been resolved by computer modeling and scanning mutagenesis.[30,31]

Mechanisms of Fibrin Selectivity

When staphylokinase is added to human plasma containing a fibrin clot, it will react poorly with plasminogen in plasma, but react with high affinity with traces of plasmin at the clot surface. At the clot surface, the plasmin–staphylokinase complex efficiently activates plasminogen to plasmin. Both plasmin staphylokinase and uncomplexed plasmin bound to fibrin are protected from rapid inhibition by α_2-antiplasmin, whereas their unbound counterparts, liberated from the clot or generated in plasma, are rapidly inhibited by α_2-antiplasmin. Thereby, the process of plasminogen activation is confined 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 elsewhere.[32]

Pharmacokinetics and Thrombolytic Properties in Patients

In patients with acute myocardial infarction treated with an intravenous infusion of 10 mg staphylokinase over 30 minutes, staphylokinase-related antigen disappeared from plasma in a biphasic mode with a $t_{1/2\alpha}$ of 6.3 minutes (mean ± SD) and a $t_{1/2\beta}$

of 37 minutes, corresponding to a plasma clearance of 270 ml/minute.[33] This short, initial half-life and rapid clearance would seem to predestine staphylokinase for administration by continuous infusion or a double bolus injection (see below). We have recently been able, however, to reduce the clearance five-fold to 30-fold by selective chemical substitution with single polyethylene glycol molecules with M_r 5,000 to 20,000, as demonstrated in experimental animal models and in pilot studies in patients with acute myocardial infarction.[34]

Recombinant staphylokinase was compared to accelerated, weight-adjusted alteplase in two open, randomized studies, each involving 100 patients with acute myocardial infarction.[32] In both studies, recombinant staphylokinase was found to be at least equipotent to alteplase in terms of complete arterial recanalization within 90 minutes. Staphylokinase was highly fibrin selective, as revealed by virtually unaltered levels of plasma fibrinogen, plasminogen, and α_2-antiplasmin. No strokes, allergic reactions, or other side effects were recorded. Thus, intravenous staphylokinase, combined with heparin and aspirin, is a potent, rapidly acting, and highly fibrin-selective thrombolytic agent in patients with acute myocardial infarction.

Recombinant staphylokinase variants have been infused intra-arterially as a 2 mg bolus followed by infusion of 1 mg/hour in 178 patients with angiographically confirmed peripheral arterial occlusion.[35] Recanalization within 48 hours was complete in 148 patients (83%), partial in 23 (13%), and absent in 7 (4%) patients. Major hemorrhagic stroke occurred in 3 patients and was fatal in 2. Blood transfusion was required in 15 patients (8.4%). One-year follow-up in 161 patients revealed amputation-free survival in 138 (86%).

Immunogenicity

Levels of preformed anti-staphylokinase antibodies in the general population are lower than those of antistreptokinase antibodies, whereas the current clinical experience in over 300 patients suggests that major allergic reactions to staphylokinase are rare.[32] Most patients, however, develop high titers of neutralizing specific IgG after infusion of staphylokinase, which would predict therapeutic refractoriness upon repeated administration.

Efforts have been undertaken to reduce the immunogenicity of staphylokinase by site-directed mutagenesis. Wild-type staphylokinase (SakSTAR variant[36]) was found to contain three nonoverlapping, 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.[37] Two variants were identified, one with Lys35, Glu38, Lys74, Glu75, and Arg77 substituted with Ala and the other with Lys74, Glu75, Arg77, Glu80, and Arg82 substituted with Ala, which did not recognize approximately one-third of the antibodies elicited in patients by treatment with wild-type staphylokinase. In patients with peripheral arterial occlusion given intra-arterial doses of 6.5 mg to 12 mg of compound, these variants induced significantly less neutralizing antibodies and staphylokinase-specific IgG than wild-type staphylokinase. Thus, these variants provided proof that reduction of the immunogenicity and immunoreactivity of recombinant staphylokinase in humans by protein engineering is feasible.

In an effort to optimize the activity/antigenicity ratio, a comprehensive site-directed mutagenesis study was carried out. Therefore, over 350 plasmids-encoding staphylokinase mutants were constructed, expressed in *E. coli*, and the expression products were purified and characterized.[34,37] A comprehensive analysis of combination variants led to the identification of staphylokinase (E65D, K74R, E80A, D82A, K130T, K135R)[2]* with intact, specific activity and that bound less than 50% of the antibodies of pooled plasma of 40 patients treated with wild-type staphylokinase. Intra-arterial administration of this variant in 18 patients with peripheral arterial occlusion induced complete recanalization in 16, but significantly less circulating neutralizing antibodies after 3 to 4 weeks than with staphylokinase. Overt neutralizing antibody induction (>5 µg compound neutralized per milliliter plasma) was observed in 56 of the 70 patients (80%) given wild-type staphylokinase, but only in 5 of the 18 patients treated with staphylokinase (E65D, K74R, E80A, D82A, K130T, K135R). In a final round of mutagenesis, approximately 100 additional plasmids were constructed, expressed, purified, and characterized, yielding staphylokinase (K35A, E65Q, K74Q, D82A, S84A, T90A, E99D, T101S, E108A, K109A, K130T, K135R, K136A, ∇137) with a maintained fibrinolytic potency and fibrin selectivity in a human plasma milieu and a markedly reduced reactivity with anti-staphylokinase antibodies in pooled, immunized patient plasma. Intra-arterial administration in patients with peripheral arterial occlusion induced SakSTAR-neutralizing activity, exceeding 5-µg/ml plasma in 2 of 7 patients. Thus, staphylokinase variants with markedly reduced antibody induction, but intact thrombolytic potency, can be generated.

Polyethylene-glycol derivatized cysteine-substitution variants for single bolus administration

Bolus administration of thrombolytic agents is becoming a preferred regimen for thrombolytic therapy of acute myocardial infarction. Derivation of proteins with polyethylene glycol (PEG) may reduce their clearance, while maintaining their specific activity. Therefore, a recombinant staphylokinase variant with reduced immunogenicity in which Ser in position 3 of the protein sequence was mutated into Cys, staphylokinase (S3C,K35A,E65Q,K74R,E80A,D82A,T90A,E99D,T101S,E108A,K109A,K130T, K135R) was derivatized with maleimide-substituted polyethylene glycol (P) with molecular weights of 5,000 (P5), 10,000 (P10) or 20,000 (P20), and characterized in vitro and in vivo.

The homogeneous 1:1 stoichiometric Cys-linked "PEGylated" variants had intact specific activities (140 to 180 kU/mg) and fibrin-selective thrombolytic potencies in a human plasma milieu in vitro (50% clot lysis with 0.32 to 0.40 µg/ml, as compared to 0.24 µg/ml for wild type staphylokinase), and their thermostability was maintained after 5 days at 37°C. PEGylation reduced the plasma clearance of an intravenous bolus in hamsters and rabbits approximately 5-fold with P5, 10-fold with P10 and 50-fold with P20. In hamsters, bolus injection induced dose related lysis of a 50 µl 125I-fibrin labeled plasma clot injected in the jugular vein: 50 percent clot lysis at

90 min was obtained with 17, 15 and 8 μg/kg, respectively, as compared to 45 μg/kg with unPEGylated staphylokinase.

Intravenous bolus injection of 5 mg of the PEGylated variants in 9 patients with acute myocardial infarction, restored TIMI-3 flow at 60 minutes in 6 patients. SakSTAR-related antigen disappeared from plasma with an initial half-life of 15, 30 and 120 min and was cleared at a rate of 70, 40 and 8 ml/min for variants substituted with P5, P10 and P20, respectively, as compared to an initial half-life of 3 min and a clearance of 360 ml/min for wild-type staphylokinase. On the basis of these results, staphylokinase(S3C,K35A,E65Q,K74R,E80A,D82A,T90A,E99D,T101S, E108A,K109A,K130T, K135R) substituted with a single polyethylene glycol molecule with a molecular weight of 5,000, linked to a cysteine residue introduced in position 3 of the amino acid sequence, has been selected for clinical development as a single intravenous bolus agent for thrombolytic therapy of acute myocardial infarction.

Plasminogen System and Tissue Remodeling

Proteinases play an essential role in cell migration and tissue remodeling, occurring in many biological processes. Proteinases degrade ECM components, a prerequisite for endothelial, smooth muscle, inflammatory, or cancerous cells to migrate to distant sites and activate cytokines or liberate sequestered growth factors. Recent gene targeting and gene transfer studies in the mouse have revealed a pleiotropic role of the plasminogen and the MMP systems in arterial neointima formation, atherosclerosis, aneurysm formation and myocardial ischemia, angiogenesis, tumor growth, metastasis, and infection. These studies will be briefly reviewed here but have been discussed in more detail elsewhere.[38]

Neointima Formation

u-PA, t-PA, and to a lesser degree, PAI-1 activity in the vessel wall are significantly increased after injury, coincident with the time of smooth muscle cell proliferation and migration, whereas expression of MMP-3, MMP-7, MMP-9, MMP-12, and MMP-13 is induced in injured, transplanted, or atherosclerotic arteries.[38] Neointima formation and neointimal cell accumulation after injury was significantly reduced in mice deficient in u-PA, plasminogen, or combined t-PA–u-PA due to impaired migration, but not proliferation, of medial and neointimal smooth muscle cells.[38] u-PAR-deficient arteries developed a similar degree of neointima formation as wild-type arteries, suggesting that sufficient pericellular plasmin proteolysis is present in the absence of binding of u-PA to its cellular receptor. Similar levels of proMMP-2 and active MMP-2, but significantly lower levels of active MMP-9, were present in arterial extracts of plasminogen deficient than in extracts of wild-type arteries. Since MMP-9 is primarily expressed by leukocytes, which are involved in the healing of the injured arteries, the lower active MMP-9 levels may contribute to the impaired medial and adventitial remodeling and the reduced neointima formation.

Atherosclerosis

Expression of t-PA, u-PA, and several MMPs in plaques is increased, but a causative role of the Plg or MMP system in atherosclerosis has not been conclusively demonstrated. No difference in the size or the predilection site of plaques have been observed between mice with a single deficiency of apoE or with a combined deficiency of apoE and t-PA, or of apoE and u-PA, suggesting that plasmin is not essential for subendothelial infiltration by macrophages. Destruction of the media with resultant, aneurysmal dilatation and rupture of the vessel wall, however, were more frequent and severe in mice lacking apoE or apoE:t-PA than in mice lacking apoE:u-PA.[39] Macrophages were absent in the media of uninvolved arteries and only able to infiltrate into and destroy the media of atherosclerotic arteries after they degraded the elastin fibers. A dramatic increase of free u-PA activity (which is minimal in quiescent arteries) was generated by the infiltrating plaque macrophages, which abundantly expressed MMP-3, MMP-9, MMP-12, and MMP-13, colocalizing with u-PA in plaque macrophages and suggesting that plasmin is a likely activator of proMMPs in vivo.[39]

Myocardial Ischemia

Recently, a mouse model of chronic myocardial infarction has been used to evaluate the role of the plasminogen system in cardiac healing (Daemen et al, personal communication). Following ligation of the left anterior descending coronary artery, wild-type or t-PA-deficient mice heal their ischemic myocardium within 2 weeks via scar formation; i.e., the ischemic myocardium becomes infiltrated by leukocytes, endothelial cells, and fibroblasts with resultant deposition of collagen. In a fraction of these mice, rupture of the ischemic myocardium occurs shortly after infarction due to excessive u-PA-generated plasmin proteolysis by infiltrating wound cells. In sharp contrast, mice lacking u-PA or plasminogen are protected against ventricular wall rupture, but fail to heal the ischemic myocardium, which remains largely devoid of infiltrating leukocytes, endothelial cells, and fibroblasts. Thus, u-PA-generated plasmin proteolysis is required for healing but needs to be carefully balanced to avoid tissue destruction and ventricular wall rupture.[38]

Angiogenesis

Migration of endothelial cells involves proteolysis of the extracellular matrix. When endothelial cells migrate, they significantly upregulate u-PA, u-PAR, and, to a lesser extent, t-PA at the leading edge of migration.[40] Although PAI-1 is also increased, its expression at different locations and times allows a net increase in fibrinolytic activity. Surprisingly, however, mice deficient in u-PA, t-PA, PAI-1, u-PAR, Plg, or α_2-antiplasmin develop normally without overt vascular anomalies.

Migration of endothelial cells alongside a denuded vessel does not require u-PA-generated plasmin, whereas invasion of endothelial cells through an anatomic barrier of ECM may (ischemic myocardium, polyoma tumor model) or may not (cornea, skin healing) require plasmin proteolysis.[38,40] Whether these differences relate to the composition or thickness of the ECM or to the expression pattern of proteinases by

endothelial cells during these different conditions remains to be determined.

Tumor Growth and Dissemination

Pericellular plasmin proteolysis has been proposed to play a role in tumor invasion and metastasis by facilitating the migration of malignant cells through anatomical barriers via degradation of extracellular matrix constituents. u-PA generally exerts a positive effect on tumorigenesis, although its mode of action (e.g., on the growth, dissemination, or progression of the tumors) may differ according to the model studied.

Based on its ability to block u-PA proteolysis, PAI-1 would be anticipated to impair tumorigenesis. The role of PAI-1 in tumor growth and metastasis remains, however, controversial, as epidemiologic studies indicate that PAI-1 is a marker of poor prognosis for survival of patients suffering from a variety of different cancers. Recent studies indicate that tumor angiogenesis (and, secondarily, tumor invasion) were markedly reduced to zero in PAI-1-deficient hosts, whereas adenoviral PAI-1 gene transfer restored the invasive behavior of the tumor cells, suggesting a role for PAI-1 in vessel stabilization.[41]

Wound Healing

Migration of keratinocytes is associated with expression of proteinases at the leading edge of their migration front. Plasminogen-deficient mice exhibited delayed and impaired closure of skin wounds, whereas combined plasminogen- and fibrinogen-deficient mice had normal wound healing,[42] indicating that fibrin mediates the effects of plasminogen deficiency. Although MMPs are likely involved in similar processes, to date, little is known about their in vivo role, as deduced from the gene targeting studies.

Infection

The expression of proteinases (in particular, of the u-PA:u-PAR system) is thought to be critical for the ability of leukocytes to degrade matrix proteins and traverse tissue planes during recruitment to inflammatory sites. u-PA has, however, also been implicated in the modulation of cytokine and growth factor expression. It is required for TNF-α expression by mononuclear phagocytes, for activation of latent TGFß-1, and may also be involved in the release of interleukin-1 (IL-1). In contrast to wild-type mice, u-PA-deficient mice were unable to mount an adequate pulmonary inflammatory response to a challenge with the nonlethal 52D *Cryptococcus neoformans* pathogen, which disseminated widely and ultimately infected the brain, leading to death. This pattern of wide dissemination and death with strain 52D has only been seen in profoundly immuno-incompetent mice.[38]

A number of invasive bacteria can interact with the host plasminogen system by expressing endogenous PAs and by binding plasminogen directly through bacterial cell-surface receptors, allowing them to utilize the PAs of the host for activation. Studies in plasminogen-deficient mice indicate that plasminogen is required for efficient dissemination of the spirochete *Borrelia burgdorferi* within the tick and for enhancement of spirochetemia in mice.[43] A similar requirement of host-derived plasminogen by *Yersinia Pestis* for its dissemination was recently reported. Bacterial strains expressing a PA (*pla*$^+$) escaped elimination by the host immune system and were almost a 1,000,000-fold more pathogenic than *pla*$^-$ strains (not expressing such a PA) in wild type, but not in plasminogen-deficient hosts.

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