Annu. Rev. Pharmacol. Toxicol. 1989. 29:323–41

THROMBOLYTIC THERAPY IN ACUTE MYOCARDIAL INFARCTION

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INTRODUCTION

Following the Federal Drug Administration approval of intravenous streptokinase for the treatment of acute myocardial infarction in May, 1987, and intravenous recombinant tissue plasminogen activator (rt-PA) for the same indication 6 months later, the medical community has embraced thrombolytic therapy as an effective intervention in this clinical condition. Recently it has been estimated that 2000 such patients per week are receiving rt-PA alone and this number is expected to grow. This wave of enthusiasm must be qualified by a host of important, currently unanswered, questions that may be crucial to designing optimal therapy. These questions relate to which agent displays the best combination of safety, efficacy, and cost, what is the maximum duration between onset of symptoms and treatment before the potential benefits of thrombolysis disappear, what is the ideal dose and whether this is best expressed in invariant terms or on a weight-adjusted basis, what patient characteristics are most strongly predictive of serious bleeding complications, and how to devise newer thrombolytic agents with enhanced potency and fewer risks. While this review touches on these and other clinically relevant topics, it concentrates in particular on basic principles of fibrinolysis which may be used by clinicians and others to comfortably administer this new therapy and to better appreciate new developments in a rapidly changing field. Acquisition of this knowledge is additionally important because it is from fundamental biochemical and pharmacologic information that major new advances will arise.

0362-1642/89/0415-0323$02.00

HISTORY

It is important to appreciate that thrombolytic agents, which first gained strong popularity in the United States by 1980–1981, are neither new nor revolutionary. Indeed, among the rapidly growing numbers of cardiovascular drugs, thrombolytic agents, particularly streptokinase, are old-timers. Streptokinase was initially described in 1933 (1), and was first given to a human in 1949 (2) and to patients with acute myocardial infarctions in 1959 (3, 4). Urokinase was first partially isolated and described in 1947 (5) and the new and revolutionary "second generation" agent tissue plasminogen activator was described in detail in 1947 (6). In a paper published in 1959 (7) a streptokinase plasmin mixture was administered to dogs through a catheter in the vicinity of an experimental thrombus in a coronary artery. After 20 min the clot had dissolved completely and reperfusion of the arterial segment distal to the occlusion had been established. In the same study histologic data demonstrated the beneficial effects of such thrombolytic therapy, including a reduction in the extent of focal necrosis.

PATHOPHYSIOLOGICAL CONSIDERATIONS

Although such promising information on the impact of fibrinolytic therapy in acute MI was published 30 years ago (3, 4, 7), it was not then accepted as a form of treatment. This reflected the controversial status of two fundamental issues. First, pathologists had debated for many years the relevance of coronary artery thrombosis to the process of acute myocardial infarction. In 1912, Herrick (8) stated that a fibrin thrombus was the precipitating cause of an acute MI. This feature was demonstrated only variably in subsequent autopsy series ranging between 21% (9) and 93% (10). Further, the possibility that coronary artery thrombus might be a consequence rather than a cause of acute MI was suggested by Erhardt et al (11).

It was not until the late 1970s that acute angiographic studies by Blanke et al (12) and DeWood et al (13) established that intracoronary thrombi occurred with a frequency of 80–90%, and that these were demonstrable shortly after the onset of ischemic symptoms. It was also established that 20–40% of the thrombi resolve spontaneously within 1–4 days. This accounts in part for the wide variation in the incidence of coronary fibrin thrombi observed in earlier autopsy series. It appears, however, that in some patients vasospasm and/or expansion of an atheromatous plaque by intralesional hemorrhage may also contribute to coronary occlusion and acute myocardial infarction.

The second long debated issue was the extent to which myocardium deprived of blood flow can be salvaged. The prevalent early opinion was based on the classical experiments of Tennant et al (14) and Blumgart et al

(15). They demonstrated that a temporary coronary occlusion in dogs maintained for more than 30–45 min resulted in irreversible myocardial damage similar to a permanent occlusion. It was not until the late 1970s that it was shown (16–18) in experiments in dogs that necrosis of ischemic myocardium is progressive, being complete only after 4–6 hr. It was also noted in these studies that myocardial viability varies widely depending on the anatomy of the coronary arteries and of the available collateral circulation. These canine experiments appear to translate well into human pathology. Although salvage of ischemic myocardium is possible in many patients with thrombolytic therapy, the variability in response between patients is considerable.

BIOCHEMISTRY OF THE FIBRINOLYTIC ENZYME SYSTEM

A thorough discussion of the fibrinolytic enzyme and the consequences of its activation provides an important framework for the subsequent consideration of thrombolytic therapy.

A simplified diagram of the fibrinolytic enzyme as it is understood today is depicted in Figure 1. The system consists of (*a*) activators, (*b*) the serine protease zymogen, plasminogen, (*c*) the activated serine protease, plasmin,

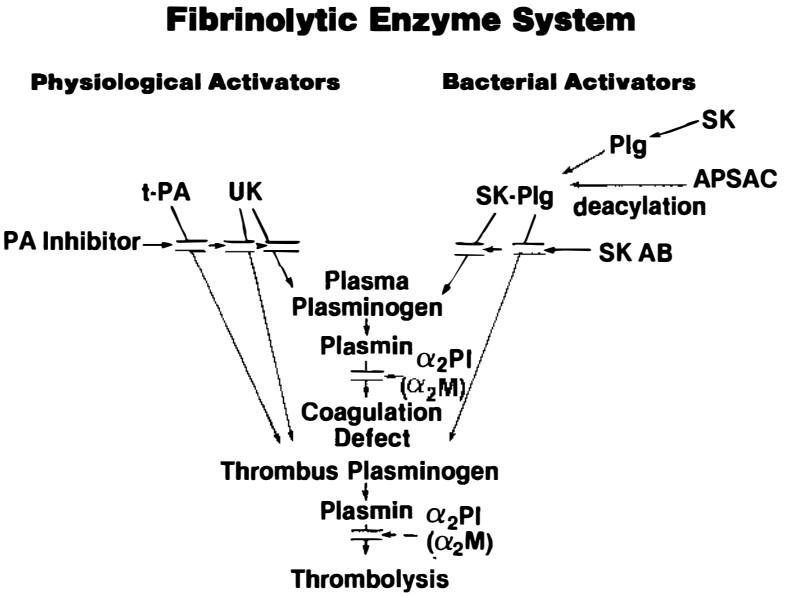

Figure 1 A scheme of the major components of the fibrinolytic enzyme system and their interaction. For details, see text.

(*d*) activator inhibitors and (*e*) plasmin inhibitors. The naturally occurring activators are tissue plasminogen activator (t-PA) and urokinase (UK), also referred to as urokinase type plasminogen activator (u-PA). While urokinase can be isolated in two forms, single-chain (scu-PA) and two-chain (tcu-PA), the term urokinase is generally used to refer to the latter moiety and is so used in this chapter. It is important to note that the two forms of u-PA activate plasminogen through fundamentally different mechanisms.

The physiologic activators are synthesized in and secreted by endothelial cells. These are inhibited primarily by newly discovered plasminogen activator inhibitors (PAI). The major one, PAI-1 (19–22), is synthesized in and secreted by endothelial cells and megakaryocytes. A second inhibitor, PAI-2 (23, 24), is synthesized in placental tissues as well as monocytes-macrophages. Others include an inhibitor directed mainly against activated protein C (25, 26), the C_1-esterase inhibitor (27), $alpha_2$ plasmin inhibitor (also called $alpha_2$ anti-plasmin) (28), and $alpha_2$ macroglobulin (29). The major PA inhibitors PAI-1 and PAI-2 are found in plasma at varying concentrations. They exist in platelets at high concentrations and are released during platelet activation (30, 31). PA inhibitors are found in increased concentrations during the third trimester of pregnancy (32), in patients with thromboembolism, and in patients with conditions predisposing to thromboembolic disease (33, 34). Thus the response to t-PA or UK may vary from patient to patient depending on the level of plasminogen activator inhibitor.

In addition to these endogenous plasminogen activators, several bacterial activators have been identified, the most prominent of which is streptokinase. Streptokinase itself has neither protease nor esterase activity but when it interacts with human plasminogen a 1:1 stoichiometric complex is formed and the proteolytically active serine of plasmin exposed; this complex activates plasminogen (35, 36). The streptokinase plasminogen complex is inhibited by streptokinase neutralizing antibodies (see below), but is not appreciably inhibited by the physiological PA inhibitors.

A special form of streptokinase plasminogen activator is prepared from equimolar complexes of streptokinase and human plasminogen in which the catalytic center of the plasminogen molecule is bound to an acyl group (37), rendering the complex inactive. In aqueous solution the acyl group is hydrolyzed and catalytic activity is regained. The rate of hydrolysis depends on the acyl group used. With the p-anisoyl derivative (anisoyl plasminogen streptokinase activator complex, APSAC) the in vivo deacylation half-time is about 90–110 min (38). Thus, APSAC can be considered a sustained release streptokinase activator preparation.

All plasminogen activators act on plasminogen by cleaving the $arginine_{560}$-$valine_{561}$ bond to produce plasmin. Plasmin is a nonspecific trypsin-like protease that proteolytically degrades fibrin in addition to several plasma

clotting factors. The major plasmin inhibitor is $alpha_2$ plasmin inhibitor ($alpha_2$ PI) (39, 40, 41). Others include $alpha_2$ macroglobulin ($alpha_2$ M) (42), C_1 esterase inhibitor, $alpha_1$ protease inhibitor, and antithrombin III (ATIII) (43, 44).

Among the plasminogen activators in clinical use there are fundamental differences between the older agents, streptokinase and urokinase, and the more recently introduced t-PA. SK and UK activate plasminogen adsorbed to the intravascular fibrin thrombus as well as circulating plasma plasminogen. As shown in Figure 2, the activation of thrombus plasminogen results in "thrombolysis from within" (45), whereas the activation of plasma plasminogen results in temporary hyperplasminemia and a profound multifactorial coagulation defect (46), including decreased concentrations of fibrinogen factor V and factor VIII. In addition, fibrinogen degradation products (fragments X, Y, & D) increase and inhibit fibrin polymerization. In contrast t-PA is relatively fibrin-specific. Its affinity for plasminogen adsorbed to the intravascular thrombus is substantially greater than that for plasma plasminogen. Consequently, t-PA tends not to activate plasma plasminogen to any significant extent. At intravenous doses sufficient to achieve coronary thrombolysis, however, sharply decreased levels of fibrinogen have been observed, albeit much less frequently than with SK and UK. Single chain urokinase plasminogen activator (scu-PA) also demonstrates thrombus specificity,

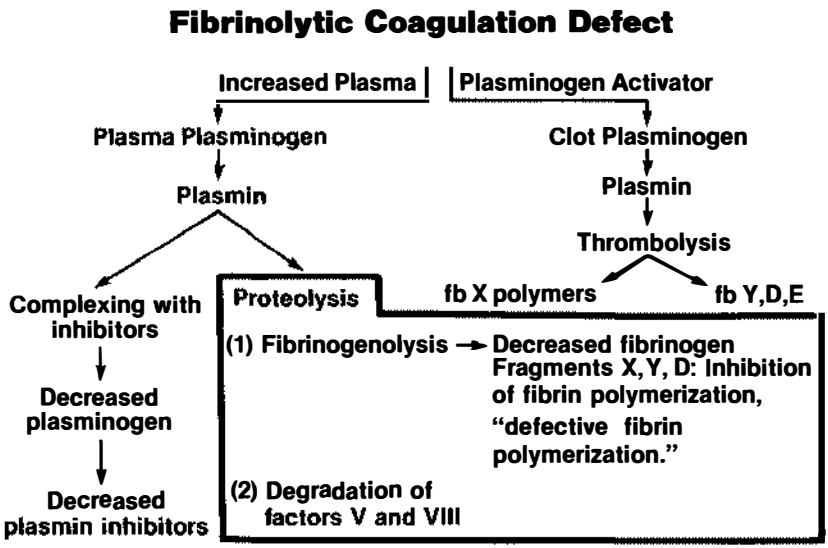

Figure 2 The major steps in the development of the fibrinolytic coagulation defect. For details, see text.

although its mechanism is unclear. One school of thought (47) maintains that scu-PA circulates in a complex with an unidentified inhibitor that dissociates from scu-PA once adsorbed to the thrombus surface. According to this theory, scu-PA is catalytically capable of converting plasminogen into plasmin. A second hypothesis (48) maintains that scu-PA is the inactive zymogen incapable of activating plasminogen: once adsorbed to the fibrin surface, however, it is converted to two chain u-PA (UK), probably by plasmin. Catalytically active UK then rapidly converts thrombus plasminogen into plasmin, resulting in thrombolysis.

The coagulation defect and other consequences of SK, UK or APSAC infusions are depicted in Figure 2. As mentioned, these activators have two points of attack: they will activate plasminogen adsorbed to fibrin, resulting in thrombolysis and fibrin degradation products, and circulating plasma plasminogen, converting it into plasmin. Most of this plasmin complexes with plasmin inhibitors to form neutralized complexes, although some remains active in the circulation. The net result is a decrease in both circulating plasminogen and plasmin inhibitors. Importantly, in sufficient quantity SK or UK can severely decrease plasminogen to undetectable levels.

As shown by Johnson & McCarty in 1959 (49), this can seriously interfere with thrombolysis. These investigators performed experiments in which thrombi were formed in superficial arm veins of human volunteers. When SK was given in doses sufficient to reduce circulating plasminogen levels to zero or near zero, most thrombi did not dissolve, or dissolved only to promptly reform. In contrast, when SK was given in doses that maintained circulating plasminogen levels at greater than 20% of the pre-infusion level, all thrombi readily underwent lysis and did not reform. It is our belief (50) that these classic experiments have direct bearing on the varying results obtained with rapid SK infusions in acute MI (see below).

A pivotal problem for thrombolytic therapy in general is bleeding associated with treatment. It is important to appreciate that for all such agents fibrinolysis will occur not only at the target thrombus but also at the sites of hemostatic plugs anywhere in the circulation. This accounts for the very high incidence of serious hematomas at venous and arterial puncture sites and is unavoidable with the infusion of any plasminogen activator. Predictably, the more fibrin specific activators t-PA and scu-PA produce extensive puncture site hematomas at a frequency equaling that seen with UK and SK.

It is noteworthy that blood coagulation accompanies, to varying degrees, activation of the fibrinolytic system by SK, UK, and t-PA. This is evidenced by significant elevations of fibrinopeptide A, which is cleaved from fibrinogen by thrombin (51). In addition, activation of platelets is suggested by a sharp increase in platelet thromboxane A_2 generation in patients undergoing thrombolytic therapy (52). The ultimate balance between anticoagulant and

procoagulant effects induced by fibrinolysis remains to be completely discerned. Such information may be critical to the clear understanding of both bleeding complications on the one hand, and reocclusion of the infarct-related artery on the other.

PHARMACOLOGY AND CLINICAL DATA: INDIVIDUAL THROMBOLYTIC AGENTS

Streptokinase

There are problems related to the use of SK unique to this drug. Streptokinase is a bacterial protein purified from Lancefield group C beta-hemolytic streptococci. The marketed products are homogenous by physico-chemical analyses but not by immunological criteria. The protein, not surprisingly, is antigenic in man. All individuals examined to date have streptokinase neutralizing antibody in the circulation at varying titers. If a patient is treated with SK, this results within 14–21 days in a sharp increase in SK neutralizing antibody. High titers of antibody are demonstrable in these patients for at least 6 months and up to several years. Even after antibody titers have returned to normal, a repeat infusion of SK may result in an anamnestic response and precipitous increases in antibody titers. Streptokinase antibodies in a patient given streptokinase are capable of neutralizing up to several million units of SK, which usually makes repeat treatment with SK ineffective and even hazardous (see below). Similarly, patients with recent streptococcal infections have circulating SK neutralizing antibodies that can immediately neutralize in excess of one million units of SK. Allergic-anaphylactic problems are also encountered (summarized in 50). These include skin allergies in 5–10% of patients, frank anaphylactic reactions in about 1–3% of cases, transient or prolonged hypotension, pyrogenic reactions with temperatures in excess of 40°C, occasionally angioneurotic edema (with lethal outcomes reported in five cases), and, rarely, hypersensitivity reactions. In a few such cases, these reactions have closely resembled classical acute rheumatic fever with unquestionable myocardial involvement. This potential for allergic-anaphylactic reactions prompts many physicians to pretreat patients with a large dose of intravenous corticosteroids prior to SK treatment. The benefits of such therapy, however, have never been clearly proven.

The clearance half-life of SK is variably estimated at 10–18 min. With the administration of even large quantities, activity is no longer demonstrable within 1 hr after stopping the infusion.

From the late 1950s through the late 1970s, SK or UK was given to patients with acute MI in moderately high doses over 12–24 hr, typically without regard to the interval between the onset of symptoms and the beginning of therapy. Several thousand patients were enrolled in these trials, summarized

by Duckert (53) and Sherry (54), and although data from early trials were encouraging, subsequent controlled trials conducted in a CCU environment failed to demonstrate any benefits of thrombolytic agents over placebo controls. Since the early 1980s, SK has been administered through the intracoronary route in moderate doses over 60–90 min. With sufficiently early treatment (i.e. less than 3–4 hr interval between onset of symptoms and beginning of therapy), it has been possible to demonstrate not only reperfusion, but reduction of infarct size and improvement of myocardial performance when compared to placebo controls (50). The intracoronary route of thrombolytic therapy is still used occasionally today, but is not practiced widely because of its high price, the obvious need for a well-equipped catheterization laboratory, highly trained personnel and, especially, its substantial time requirements. It is universally agreed that time is of the essence when thrombolytic therapy in acute MI is contemplated. This appears particularly true with SK, where thrombolysis is dependent on the age of the clot and is substantially impaired with thrombi older than three hours. In addition, the earlier the treatment is started the better the chances for salvaging ischemic myocardium, and improving both left ventricular function and survival. The intracoronary route, compared with intravenous administration, typically mandates at least one additional hour for initiating treatment, an hour that can cost dearly in terms of irreversible damage to the ischemic myocardium. Today the most widespread treatment is a rapid infusion of intravenous SK. The recommended dose is 1.5 million units given over one hour, a dose that frequently reduces circulating plasminogen levels to near zero (50).

Intravenous SK administration has produced widely varying clinical results with reperfusion being established in 10–65% in 52 studies (summarized in 50). However, in five recent large studies, all lacking angiographic assessment of reperfusion, SK was clearly beneficial in reducing mortality and/or improving myocardial performance. The collaborative Italian study GISSI (55–56) examined the early and one year mortality rates in almost 12,000 patients randomized to receive SK or placebo and admitted to the CCU within 12 hr after the onset of symptoms. Overall, the hospital mortality of SK recipients was decreased 13% compared to controls. When treatment was started within 3 hr after the onset of symptoms mortality was reduced by 23%, and if treatment was started within 1 hr after symptoms, the reduction was 47%. This survival advantage was marginal at 3–6 hr after onset of symptoms and became insignificant at greater than 6 hrs. Improvements in survival were preserved at one year after admission. However, a significantly higher incidence of reinfarction during the hospital stay and during the one year follow-up period was noted among SK recipients compared to controls. A second collaborative effort, the ISAM study group (57), performed a similar trial in 1,741 patients. No statistically significant in-hospital difference in

mortality between the SK group (5.2%) and the placebo group (6.5%) was documented. There was, however, convincing evidence of smaller infarcts and higher global and regional ejection fractions in the SK group. A third study from New Zealand (58) enrolled 219 patients presenting within 4 hr of infarction. In this study in-hospital mortality was lower, 2.5% vs. 12.9%, and the left ventricular ejection fraction was significantly improved in the SK group compared to the placebo controls. The Western Washington Trial (59, 60) randomized 368 acute MI patients to receive intravenous SK or placebo within 6 hr of the onset of symptoms. Significantly improved long-term survival was seen in SK-treated patients with anterior MIs. There was not, however, statistically significant improvement in long-term survival in patients with inferior MI or in 14-day survival in patients with either anterior or inferior MI. Finally, a very large collaborative trial, ISIS II, has been performed in Europe and presented orally but not published[1,2]. Approximately 17,000 patients were randomized within 24 hr after the onset of symptoms to placebo, SK, placebo + aspirin (160 mg daily) or SK + aspirin. SK alone reduced the 5 week mortality by 21% and aspirin alone reduced mortality by 19%. In the group treated with both SK + aspirin, 5 week mortality decreased by almost 40%. This study is notable for its size, the provocative data on both SK and aspirin, and the fact that mortality benefits were demonstrable in patients treated as late as 13–24 hr after the onset of symptoms. It is compromised, however, by loosely defined eligibility criteria, which may have resulted in the inclusion of a large number of patients with conditions other than acute MI. The published report should provide important clarification of trial design, patient entry criteria, and data analysis.

Anisoylated Plasminogen-Streptokinase Activator Complex (APSAC)

The coupling of streptokinase-plasminogen to p-amidinophenyl p-anisate, HCl produces a drug with several theoretical advantages. The temporary masking of the catalytic center of the activator complex does not interfere with the capacity of the molecule to bind to fibrin since the fibrin binding sites of plasminogen ("the kringle domain") are located in the plasminogen molecule well separated from the catalytic site. Thus, relative fibrin specificity should be conferred: this has been confirmed in in vitro experiments (61) and in animal experiments (62) where APSAC proved more efficient in lysing experimental thrombi and caused less fibrinogen depletion than streptokinase-plasmin. However, at doses given to man sufficient to cause coronary reperfu-

[1]Annual meeting of American College of Cardiology, Atlanta, Georgia, March 24–30, 1988.

[2]Ninth International Congress of Fibrinolysis, Amsterdam, Netherlands, June 27–July 1, 1988.

sion (30 mg as a bolus injection), APSAC causes considerable fibrinogen depletion and a rate of bleeding complications not apparently different from streptokinase (63). Further, hypotensive reactions have occasionally been reported in patients (64).

Although APSAC has been shown to bind less streptokinase neutralizing antibody than SK (65), the incidence of clinical allergic-anaphylactic reactions is similar to that observed with SK (66). The prolonged t½ of approximately 90–110 min for APSAC permits bolus administration and may reflect slower clearance by the liver and/or less neutralization by plasma inhibitors that cannot interact with the complex until deacylation occurs (67).

APSAC therapy, when instituted early after the onset of symptoms, has in several clinical studies led to a reperfusion rate of 60–65% and a patency rate of 70–80% (summarized by Anderson, 66). In a recent placebo-controlled study (68), APSAC administration in acute MI resulted in an impressive 47% reduction in mortality.

Urokinase (UK)

Urokinase, the first of the naturally occurring and direct plasminogen activators used for therapeutic purposes, was originally prepared from urine, but is currently being produced from primary cultures of fetal kidney cells and through recombinant DNA technology. Commercial urokinase is either high molecular weight or low molecular weight, the latter form being derived from the former by proteolysis in the culture medium. Both forms are highly active and have been equally effective in clot dissolution in patients (69). The t½ of urokinase in vivo is estimated at 12–20 min (70). Urokinase is not antigenic in man and does not cause acute reactions (hypotension) when administered rapidly. Only limited clinical data on the short-term intravenous administration of UK in acute evolving myocardial infarction are available (71, 72). The incidence of reperfusion was 66% with UK as opposed to 78% observed with intracoronary SK or intravenous recombinant t-PA. Limited evidence for improvement of left ventricular function following intravenous UK has also been presented (71). The hemostatic defect and incidence of bleeding with UK is comparable to that produced by SK and APSAC (71, 72).

Tissue Plasminogen Activator (t-PA)

Although t-PA was discovered almost five decades ago and its unique fibrin specific properties first established in the early 1970s (73, 74), concerted research efforts on this enzyme began only when Rijken in Collen's laboratory (75) demonstrated that t-PA was secreted in substantial quantities by a malignant human melanoma cell line (Bowes) and described a convenient procedure for the complete purification of the activator from conditioned tissue culture medium. In 1983 (76), a collaborative effort between Genen-

tech scientists and Collen's group resulted in the cloning and expression of t-PA in mammalian as well as prokaryotic cells. The material marketed today is recombinant t-PA produced in mammalian cells. This preparation is largely in single-chain form. When exposed to plasmin this molecule is rapidly converted to a two-chain heterodimer. The high fibrin selectivity of t-PA is thought to reflect the formation of a specific ternary complex between fibrin, plasminogen, and t-PA. Without the fibrin component, the t-PA molecule is an inefficient activator of plasminogen. In formal kinetic experiments with purified components, the catalytic efficiency of t-PA in the presence of fibrin is approximately 500-fold greater than in the absence of fibrin (77). However, other surfaces, e.g. those of activated platelets and of endothelial cells, are capable of assembling t-PA and plasminogen in the correct steric configuration to efficiently promote plasmin generation (78, 79).

Recombinant t-PA has been highly publicized as being more effective and safer than the less clot-selective SK, APSAC and UK agents. Unfortunately, observations reported to date do not support this contention. The t½ of recombinant t-PA is short, approximately 5 min (80). Sustained infusions (approved dose = 100 mg) of the agent therefore have to be administered to insure reperfusion in the majority of patients. After open-label studies with t-PA had produced encouraging results (81, 82), two multicenter controlled trials comparing t-PA and SK in patients with acute MI were published in 1985. The American TIMI trial (83) compared a 3 hr infusion of recombinant t-PA (80 mg) to a 1 hr infusion of streptokinase beginning on average 4.8 hr after the onset of symptoms. T-PA was associated with reperfusion of the infarct-related artery in 62% of the 118 patients in this group. In 122 patients receiving streptokinase the infarct-related artery was reperfused in only 31%, a statistically significant difference. Bleeding complications were similar in the two groups. The major criticism of the TIMI trial is that it fails to take into consideration the time between onset of symptoms and therapy. It had previously been established (84) that short duration intravenous streptokinase, when administered within the first 3 hr after the onset of the ischemic event, produces reperfusion in 75% of patients, but that this figure decreases to 38% when the therapy is delayed until the 3–6 hr period. In contrast, reperfusion with t-PA appears to be relatively independent of the age of the coronary thrombus.

The European cooperative study (85) of 123 patients, published simultaneously with the TIMI study, compared recombinant t-PA with SK administered an average 3 hr from the onset of symptoms. Patients who received t-PA had a higher reperfusion rate than did the SK treated patients (70 vs. 55%), but this difference was not statistically significant. In additional trials subsequent to 1985, patency rates with t-PA have been 61% (86), 75% (87), and 88% (88). Bleeding complications in the European study, like the TIMI study, were similar in the t-PA and SK-treated groups.

Only one published study to date (89) examines the beneficial effects of t-PA on myocardial performance in acute MI. In this study the left ventricular ejection fraction, measured by radionuclide ventriculography 10 days after hospital admission, improved by 3.5% in 72 patients given t-PA and decreased by 4.7% in 66 patients given placebo. These differences are statistically significant. No published study to date has examined whether t-PA lowers the early and late mortality rate in patients with acute MI. This is in contrast to streptokinase where substantial reductions in mortality have been clearly established. (See above).

The rate of bleeding complications with thrombus-specific t-PA has been high. Contributing to the high incidence of bleeding has been the concomitant administration of heparin and the frequent use of invasive procedures. In the first TIMI trial (83), mild bleeding problems occurred in 47% of patients in the streptokinase group and 43% in the t-PA group. Gastrointestinal bleeding was noted in 10 and 6%, respectively. In later studies (86–88), minor hemorrhage has occurred in up to half of the patients and in one study (87) serious bleeding episodes requiring transfusion were encountered in 18%. Of note, in studies performed with a 150 mg dose of t-PA 1.6% of patients (16 of 1,014) experienced intracranial hemorrhage, whereas at a 100 mg dose 0.6% of patients (84 of 1,452) had this complication. Both doses were associated with similar patency rates (90).

The reocclusion rate with t-PA is quite substantial, ranging from approximately 10–33% in four studies (81–83, 91). Whether these reocclusion episodes reflect the potential for activation of blood coagulation and platelets concomitantly with activation of the fibrinolytic enzyme system is not known. That they occur in spite of heparin therapy strongly suggests the need for therapeutic alternatives to this anticoagulant.

Single-Chain Urokinase-Type Plasminogen Activator (scu-PA)

Clot-specific thrombolysis by natural or recombinant scu-PA has been demonstrated in animal models of venous thrombosis and coronary artery thrombosis (92). The reported t½ in man of scu-PA is approximately 3–20 min, however full thrombolytic potential is reached only after a considerable and variable latent interval (70). In patients with acute MI, intravenous infusion of 40–70 mg over one hour resulted in coronary artery reperfusion in 75% of the patients, but pronounced fibrinogen depletion occurred in 25% (93).

Synergistic combinations of t-PA with UK and scu-PA have worked well in animal experiments (94) and in preliminary studies in patients with acute MI (95, 96). The ultimate efficacy and safety of such combination regimens and their synergistic potential remains to be established by studies involving larger numbers of patients.

FUTURE AGENTS: MUTANT AND HYBRID PROTEINS

With the rapid advancement in recombinant DNA technology it is possible to structurally alter plasminogen activators and to produce plasminogen activator hybrids. Such efforts have been very useful in elucidating structure function relationships of plasminogen activator molecules, but the ultimate aim, that of creating superior therapeutic agents, remains elusive. Attempted modifications of t-PA include deletion of the N-terminal "finger" and/or "EGF like domains" and/or the "kringle 1" (97–100), the removal of one or more of the three carbohydrate side chains through point mutations of asparagine residues to which these chains are attached in N-linkage (97, 101), and point mutations of residue arg_{275} resulting in a molecule that does not undergo the normal conversion from the single-chain to the two-chain form (102). These approaches can result in molecules with significantly prolonged biological half lives and, occasionally, improved fibrin specificity (103, 104). Catalytic efficiency, on the other hand, has not thus far been improved by these approaches. The deletion of two major domains in t-PA, "kringle 1" and "kringle 2" produces a thrombus-specific molecule that interacts poorly with its major inhibitor PAI-1 (105, 106). Several hybrids have been reported (107, 108) that combine the C-terminal serine protease domain of u-PA responsible for plasminogen activation with the heavy chain of t-PA responsible for the high fibrin dependence of that molecule. These hybrids usually exhibit higher catalytic efficiency than t-PA but show significantly decreased fibrin binding and thrombus-specificity than the parent t-PA molecule. Hybrid proteins combining the catalytic domain of u-PA or t-PA with monoclonal antibodies of high fibrin-specificity have also been constructed. These have been demonstrated in preclinical studies to possess very high thrombus-specificity, a prolonged $t_{1/2}$, and greatly enhanced thrombolytic potential compared with t-PA or u-PA with little or no accompanying fibrinogen depletion (109).

CONCLUSIONS AND FUTURE CONSIDERATIONS

There is little doubt that aggressive thrombolytic therapy in acute myocardial infarction has opened an exciting new chapter in therapeutics that appears to provide substantial and lasting benefits to patients. Many major questions alluded to at the beginning of this chapter, however, remain unresolved at this time. For example, it is not clear whether the very expensive t-PA offers clear benefits over the much cheaper SK. Although SK under specific experimental conditions of recently published studies (50, 85) achieves reperfusion less frequently than t-PA, irrefutable evidence for reduction in mortality following acute MI has been presented for SK. Analogous data do not exist for t-PA.

The sustained release SK preparation APSAC offers the convenience of bolus administration, an incidence of reperfusion roughly comparable to that of SK, and, like SK, substantially reduced mortality in acute MI. Although t-PA creates a less severe coagulation defect than SK or APSAC, it results in a similar rate of bleeding complications. Heparin administered concurrently undoubtedly contributes to this high incidence of bleeding complications. If, as many suspect, t-PA must be given concurrently with heparin or other antithrombotic agents, then one must accept for t-PA the combined risk of both anticoagulation and thrombolysis.

The high incidence of reocclusion with t-PA is another serious problem clearly not resolved by the concurrent use of heparin. Future research must focus on antithrombotic therapeutic alternatives to heparin, including activated protein C (110), 5-HT_2 receptor antagonists and/or thromboxane A_2 receptor antagonists (111), monoclonal antibodies directed against the platelet glycoprotein IIb/IIIa receptor (112) or fibrinogeno-mimetic peptides (113), which have all shown promise in preclinical pharmacology but remain clinically untested to date.

The creation of recombinant hybrid or mutant molecules of higher fibrin-specificity, longer biological half lives, and higher catalytic efficiency remains an attractive possibility, as do hybrid molecules combining fibrin-specific monoclonal antibodies with plasminogen activator or portions of plasminogen activator. In the final analysis, the relative value of competing agents and unique new molecules must await large scale, appropriately controlled, clinical trials.

ACKNOWLEDGMENT

We gratefully acknowledge the excellent secretarial assistance of Ms. Sharon Jackson and Ms. Carolyn Rork.

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NOTE ADDED IN PROOF

Since this manuscript was submitted for publication, the ISIS-2 trial has been published (ISIS-2 Collaborative Group. 1988. Randomized trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. *Lancet* 2:349–60). In addition, the first trial comparing t-PA to placebo in which death was the primary endpoint has also been published (Wilcox, R. G., von der Lippe, G., Olsson, C. G., Jensen, G., Skene, A. M., et al. 1988. Trial of tissue plasminogen activator for mortality reduction in acute myocardial infarction. Anglo-Scandinavian study of early thrombolysis (ASSET). *Lancet,* 2:525–30). In 5011 patients randomly allocated to receive t-PA or placebo within 5 hours of the onset of symptoms of acute myocardial infarction, 1 month mortality was reduced by 26% in the t-PA treated group. This is comparable to mortality reductions observed in both GISSI and ISIS-2.