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# Biochemical Pharmacology and Therapeutic Aspects of Thrombolytic Agents

R. FEARS

*Beecham Pharmaceuticals Research Division, Great Burgh, Epsom, Surrey KT18 5XQ, United Kingdom*







## I. Introduction

Formation of a fibrin matrix during haemostasis is part of the physiological response to vascular injury, whereas thrombosis is a pathological obstruction of blood flow, but the cellular and biochemical participants are similar. Physiologically, fibrinolysis acts to maintain vascular patency by the proteolytic degradation of fibrin; local control of fibrinolysis requires a coordinated interaction of enzymes, zymogens, and inhibitors.

Vascular disease is not a recent phenomenon and relevant observations can be traced into antiquity. For

example, Hippocrates introduced the concept of blood stasis as a pathological condition and Galen described a vascular obstruction caused by what is now recognised as a thrombus. Some of the cardinal observations that led to an understanding of thrombosis and fibrinolysis are listed in table 1. The interrelationship between clot formation and lysis can be ascribed to the central role of fibrin as product, substrate, and cofactor; the use of plasminogen activators (table 1) as pharmacological tools has contributed to many of the recent advances in thrombosis research.

Apart from the pivotal function of fibrin, there are other common features between the coagulation and fibrinolytic pathways, most notably the amplification of proteolysis by conversion of substrate proenzyme to active serine protease (fig. 1) but, also, a sharing of regulatory proteins. These mutual controls include factor XIIa, which participates in both intrinsic coagulation and intrinsic plasminogen activation, and antithrombin III, which is an inhibitor of both thrombin and plasmin (to a lesser extent). Furthermore, plasmin can digest coagulation factors in addition to fibrin. Plasminogen activators are serine proteases with restricted substrate specificity that can catalyse the hydrolysis of the Arg 561—Val 562 bond in the zymogen, plasminogen, to produce plasmin (fig. 1). Plasmin has a relatively broad, trypsin-like, specificity so that the selective physiological function of fibrinolysis requires some localisation of plasminogen activation within the fibrin mesh. The demonstration that fibrin-bound plasmin is partially protected from neutralisation by the inhibitor $\alpha_2$-antiplasmin

TABLE 1
*Key dates in thrombosis and fibrinolysis investigation*

| Investigator (yr) | Subject |
|---|---|
| **Physiology and pathology** | |
| Malpighi (1666) | Role of fibrin in blood clotting |
| Heberden (1782) | Fluidity of blood after sudden death (release of activator from endothelium) |
| Virchow (1856) | Determinants of thrombosis: vessel injury, stasis, hypercoagulability |
| Dastre (1893) | Definition of fibrinolysis |
| Morawitz (1905) | Elucidation of theory of blood coagulation |
| Nolf (1908) | Interrelationship between clot formation and lysis |
| **Biochemistry of plasminogen activators** | |
| Tillet and Garner (1933) | Identification of fibrinolytic protein in $\beta$-hemolytic streptococci |
| Christensen and MacLeod (1945) | Nature of plasminogen, plasmin, and streptokinase |
| Astrup and Permin (1947) | Discovery of tissue-type plasminogen activator |
| Williams (1951) | Identification of urokinase (urinary fibrinolytic factor described by Sahli in 1885) |

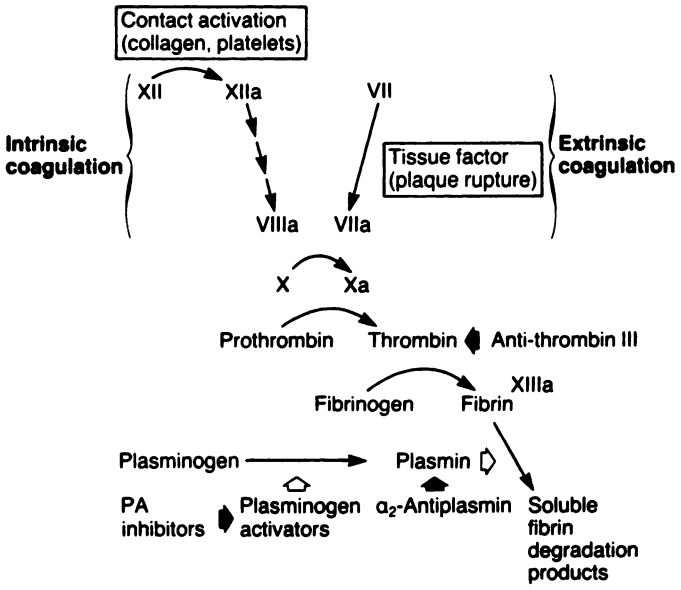


FIG. 1. Coagulation and fibrinolysis pathways. Action of principal inhibitors is indicated by ⊸. PA, plasminogen activator.

(Rákoczi et al., 1978) and that the endogenous activator, t-PA,* has a high affinity for fibrin (Hoylaerts et al., 1982) provides validation for the initial hypothesis describing physiological thrombolysis as the local activation of fibrin-bound plasminogen in an inhibitor-free environment (Alkjaersig et al., 1959).

Thrombus formation is an important factor in many clinical disorders and there is considerable current enthusiasm for the use of thrombolytic agents to treat arterial and venous thrombotic states. The clinical indication attracting the most attention is AMI in which coronary artery thrombosis leads to myocardial ischaemia and necrosis unless flow is rapidly restored. It is the purpose of this review to focus on the pharmacological approaches to AMI, the major cause of death in most industrialized countries. Both the clinical findings for the standard agents, with reference to their biochemical pharmacology, and the initial findings regarding structure-activity relationships for novel plasminogen activators in preclinical research are described. The properties of the thrombolytic agents are considered in the context of the normal regulatory mechanisms of the haemostatic and fibrinolytic pathways and with regard to the possibilities for potentiation of activity by adjunct therapies.

## II. Endogenous Plasminogen Activators

The principal endogenous plasminogen activators are t-PA and u-PA. Both t-PA and u-PA primarily act in

* t-PA, tissue-type plasminogen activator; AMI, acute myocardial infarction; u-PA, urokinase-type plasminogen activator; scu-PA, single-chain molecule of u-PA; tcu-PA, two-chain derivative of u-PA; Lp(a), lipoprotein subfraction a; SK, streptokinase; Lys, lysine; Glu, glutamic acid; Arg, arginine; Val, valine; Ile, isoleucine; APSAC, anisoylated Lys-plasminogen streptokinase activator complex; EACA, $\epsilon$-aminocaproic acid; PAI-1, plasminogen activator inhibitor type 1.

processes requiring extracellular proteolysis, but they are products of different genes and appear to have different biological functions (Bachmann and Kruithof, 1984; Verstraete and Collen, 1986). The principal action of t-PA is believed to be in fibrinolysis, although t-PA can specifically bind to other proteins and to cells (Fears, 1989a). There is some evidence for its involvement in ovulation and in prohormone processing. The activity of u-PA may contribute to a variety of biological processes involving tissue destruction and cell migration, for example, inflammatory reactions, tissue involution, and tumour invasiveness and metastasis (Blasi et al., 1987).

### *A. u-PA*

Many cells synthesise and secrete u-PA, which can exist as a proenzyme, a single-chain molecule (scu-PA), or as the two-chain derivative (tcu-PA), generated by lysis of the Lys 158—Ile 159 peptide bond. scu-PA demonstrates fibrin specificity, not by fibrin-binding but possibly either because of a competitive inhibition by a protein in plasma that is reversed in the presence of fibrin or by preferential activity on conformationally altered plasminogen bound to partly lysed fibrin (Lijnen et al., 1989). Binding of u-PA to its cellular receptor focuses proteolytic activity to the cell surface resulting, for example, in a mitogenic effect in human tumour cell lines. Antibody inhibition of u-PA activity in human melanoma cells suppresses cell proliferation and may represent a novel approach to the modulation of tumour growth in vivo (Kirchheimer et al., 1989). The significance of the recent evidence that u-PA can act directly on proteins other than plasminogen has yet to be assessed. A direct action of u-PA on fibronectin in the extracellular matrix (Gold et al., 1989) may induce loss of cellular adhesion and thus aid tumour cell invasion and metastasis.

### *B. t-PA*

Endogenous control of fibrinolysis is usually thought to reflect changes in endothelial cell t-PA activity rather than changes in u-PA turnover or the operation of the intrinsic pathway (Prowse and Cash, 1984). Net activity of t-PA is dependent on the relative rates of release from endothelial cells, hepatic clearance, and local binding to fibrin but also on the interaction with circulating inhibitors, particularly PAI-1 (Sprengers and Kluft, 1987). General features of the physiological variations in t-PA activity have been reviewed elsewhere (for example, Reiner and Bell, 1984) and the regulation of t-PA expression was reviewed recently by Gerard and Meidell (1989).

The precise control mechanisms are still poorly understood but a common response to a diverse range of mediators is represented by t-PA release from the endothelium (table 2). Experimental studies have not necessarily distinguished between an effect on t-PA synthesis de novo and release of t-PA from a tissue store. Furthermore, although a rapid response may be considered indicative of an effect on release rather than biogenesis, it is difficult to distinguish experimentally among secretion, displacement of t-PA from the cell surface, and inactivation of PAI-1. Studies (Smith et al., 1985) indicate that bradykinin may be more potent than other vasoactive agents. The mechanism could be mediated by prostaglandin synthesis or by a neural reflex, and the production of bradykinin from high molecular weight kininogen may, therefore, be an important event in the initiation of fibrinolysis. Taken together, the evidence suggests that endothelial cell release of t-PA and of PAI-1 may be controlled both by adenylate cyclase and by phosphoinositide signal transduction pathways (Francis and Neely, 1989). The similarity in response to the cytokines interleukin 1 and tumour necrosis factor-$\alpha$ (table 2) suggests that local development of inflammatory or immune processes could also be important in regulating endothelial fibrinolytic activity (Schleef et al., 1988).

TABLE 2

*Biochemical mediators of endothelial release of t-PA and PAI-1*

| Mediator | Change in activity | |
|---|---|---|
| | t-PA | PAI-1 |
| Catecholamines | ↑ | ? |
| Vasoactive agents (histamine, vasopressin, acetylcholine, serotonin, bradykinin) | ↑ | ? |
| Agents increasing intracellular cyclic adenosine monophosphate (e.g., prostacyclin, prostaglandin $E_1$) | ↓ | ↓ |
| Thrombin | ↑ | ↑ |
| Interleukin 1 | ↓ | ↑ |
| Tumor necrosis factor $\alpha$ | ↓ | ↑ |
| Endotoxin | ? | ↑ |

## III. Thrombotic Disorders

Much can be learned about the normal controls on the operation of the coagulation and fibrinolytic pathways (fig. 1) by examining the consequences of specific biochemical lesions, but because the topic has been reviewed in detail recently (Lijnen and Collen, 1989) only selected examples will be mentioned now.

Recurrent thrombosis, usually venous (primarily deep vein thrombosis and pulmonary embolism), has been associated with genetic abnormalities in circulating proteins. For example, decreased t-PA activity (decreased t-PA synthesis or increased PAI-1 synthesis), decreased antithrombin III antigen or functional activity, elevated histidine-rich glycoprotein (Engesser et al., 1987), and homozygous protein C deficiency have all been implicated. Point mutations in plasminogen (Aoki etal., 1978) and fibrinogen also may be associated with recurrent venous thrombosis.

In addition to the specific molecular defects, clinically important changes in the coagulation and fibrinolytic pathways may develop secondarily to other disease states (Reiner and Bell 1984; Kwaan 1984). Systemic changes

in the operation of the fibrinolytic pathway can occur as a result of changes in the rate of clearance of key proteins. For example, in kidney disease there may be an increased urinary loss of plasminogen (Reiner and Bell, 1984) and of antithrombin III, augmenting the tendency to fibrin deposition. In chronic liver disease, there is decreased clearance of t-PA (Francis and Feinstein, 1984) and also of factor VIII (Langley et al., 1985). A tendency to accelerated fibrinolysis in liver disease is supported by the decreased synthesis of $\alpha_2$-antiplasmin (Francis and Feinstein, 1984) and a decrease in the proportion of plasminogen rendered relatively unavailable by binding to histidine-rich glycoprotein (Gram et al., 1985).

In spite of the advances in our understanding of the biochemistry of haemostasis that have been made as a result of studying the various primary and secondary disorders, the quantitative significance of the predisposing factors to the pathogenesis of venous thrombosis remains to be defined. Thus, although patients with deep vein thrombosis can be classified according to whether the concentration of t-PA antigen was low or PAI-1 was high (Nilsson et al., 1985), the principal causative factors of thrombosis in postsurgical patients are associated with tissue factor released during tissue damage and venous stasis.

The nature of the contribution made by impaired fibrinolysis to arterial disease has been even less clear, although evidence was first provided by Virchow (table 1) and by Rokitansky in 1855, who proposed that mural deposition of fibrin was the initiating event in atherogenesis. The finding (Smith and Ashall, 1985) that plasminogen could rarely be detected in the aortic intima of patients dying from AMI indicates that there may well be a specific alteration in the balance between vessel wall and circulating pools of plasminogen. Other studies of patients with AMI suggest that circulating levels of fibrinogen and PAI-1 are elevated in comparison with control subjects (Estelles et al., 1985; Hamsten et al., 1986). Further information concerning PAI-1 and another circulating protein potentially involved in atherosclerosis and thrombosis, Lp(a), is provided in sections IV, C, 2 and IV, C, 3.

Increased fibrin formation may, therefore, be a common feature of both the underlying atheroma, developing throughout decades (Fears, 1989b), and the occlusion that is now known to precipitate AMI in the majority of patients (De Wood et al., 1980). However, the mechanism responsible for the focal initiation of coronary thrombosis remains speculative. It is likely that plaque fissuring is the ultimate stimulus to thrombotic occlusion (Ridolfi and Hutchins, 1977; Davies and Thomas, 1985), but the determinants of plaque ulceration and fissuring have yet to be understood (Fears, 1989b).

## IV. Pharmacological Properties of Plasminogen Activators Used as Therapeutic Agents

### *A. Pharmacological Manipulation of Endogenous Fibrinolysis*

The evidence from epidemiological and genetic studies showing a predisposition to thrombosis, when the potential for endogenous fibrinolysis is impaired, has led to many attempts to augment basal levels of fibrinolytic activity in high-risk patients. There are empirical findings of a variety of pharmacological effects (table 3), some simulating physiological control mechanisms (table 2). $\beta$-Agonists may induce t-PA synthesis as well as release, at least in some cell types (Conanan and Crutchley, 1983), although the impaired t-PA secretion seen in young survivors of AMI was related to a chronic exhaustion of endothelial stores in response to the high circulating concentration of catecholamines (Hamsten et al., 1985).

Few of the agents found to promote endogenous fibrinolytic activity have been of continuing interest because of the relatively small changes achieved, tachyphylaxis that occurred, and side effects produced. Manipulation of endogenous pathways is insufficient to accomplish the rapid provision of plasminogen activator activity required to restore blood flow in an occluded artery in AMI. Therefore, to lyse the coronary thrombus it is necessary to administer exogenous plasminogen activator.

### *B. Exogenous Agents*

Plasminogen activators catalyse the cleavage of the same peptide bond in the zymogen (fig. 2). Enzymatic activity is, however, influenced by many factors, including the interaction of activator with fibrin and other macromolecules, and by conformational changes within the activator in response to the action of formed plasmin. The amino acid sequences and structural properties of the endogenous activators, t-PA, scu-PA, and tcu-PA, have been well-characterised (Bachmann and Kruithof, 1984; Verstraete and Collen, 1986) and considerable experience has been gained regarding the use of pharmacological amounts of these enzymes in the treatment of AMI. Indeed, it was the seminal elucidation by Pennica and coworkers (1983) of the sequence of t-PA (alteplase, Activase) and the pioneering experimental work of Collen and colleagues (Verstraete and Collen, 1986; Collen et al., 1988) on t-PA and scu-PA that has contributed much to the excitement in this therapeutic area.

Two other thrombolytic agents have been the subject of extensive clinical evaluation. One is SK, which is not an enzyme but forms a plasminogen activator (SK-plasminogen) on stoichiometric binding (1:1) with endogenous plasminogen (aminoterminal Glu). Although SK-plasminogen was the first fibrinolytic protein to be characterised (table 1), recent research is providing new insight into the conformational properties of SK (Radek and Castellino 1989). The other plasminogen activator

TABLE 3
*Pharmacological approaches to augmenting circulating t-PA activity*

| t-PA | | PAI-1 (decreased synthesis) |
|---|---|---|
| Increased release from endothelium | Increased synthesis | |
| β-Agonists | Phytosterols: sitosterol, fucosterol | Stanozolol, danazol |
| Nicotinic acid and vasoactive analogues | Sulphonylureas: tolbutamide, chlorpropamide, glipizide | |
| Heparin and other sulphated polysaccharides | Biguanides: phenformin | |
| Desamino-8-D-arg-vasopressin | Cycloallicin | |
| Defibrotide | Clofibrate | |

currently available, APSAC (anistreplase, Eminase), a proenzyme of the SK-activator complex containing a proteolytically modified form of plasminogen, Lys-78-plasminogen (Smith et al., 1981; Fears 1989c). APSAC is produced as a stabilised acyl-enzyme using an inverse acylating agent, whereby an amidinophenyl group functions as an arginine analogue to introduce, temporarily, a substituted benzoyl group into the active centre (Smith et al., 1981). Conversion to the active enzyme occurs by hydrolytic deacylation in a sustained and controlled manner: studies of de-acylation rate by both radiometric and functional methods (Hibbs et al., 1989) estimate a half-life of approximately 105 min, unaffected by fibrinolysis or the presence of blood cells. It was envisaged (Smith et al., 1981; Ferres, 1987) that the temporary chemical protection of the catalytic centre in APSAC could yield several benefits, including prolonged generation of enzymatic activity and retardation in rate of loss of activity from the blood stream.

The primary pharmacological objective of thrombolytic therapy for AMI is the achievement of a rapid and complete lysis of the coronary thrombus; the pharmacological criteria necessary for this task have been proposed elsewhere (Ferres, 1987, 1988). Evidence will, therefore, now be discussed comparing the standard agents in terms of two desirable therapeutic attributes: bioavailability and thrombus affinity.

### C. Bioavailability

*1. Comparative pharmacokinetics.* Because mode of administration and duration of activity will be influenced by the residence time of a plasminogen activator in the circulation, the measurement of pharmacokinetics is an important aspect of the clinical pharmacology of thrombolytic agents and of the optimisation of their use in therapy. Clearance from the circulation is subject to various determinants (fig. 3): activity is lost primarily either by hepatic uptake (for t-PA and u-PA), by local inactivation in the circulation by inhibitor complex formation, or by proteolysis (SK-plasminogen).

The various published methods for pharmacokinetic analyses include radiometric and immunodetection assays and assays based on measuring enzymatic activity with regard to amidolytic (chromogenic substrate) or fibrinolytic end points. The result obtained is dependent on the type of method chosen (Fears et al., 1989a); an assay based on the dissolution of fibrin can be considered as the reference method, if calibrated. With such methods, the euglobulin fraction is prepared from plasma to exclude the continuing influence of inhibitors, but it is also important to dilute the euglobulin fraction sufficiently to nullify any variable contribution by plasma plasminogen and plasmin to the lytic end point (Fears et al., 1990b). Such precautions are of particular importance when measuring the lysis of bovine fibrin as an end point because bovine plasminogen may be relatively poorly activated and artifacts can be obtained (Col et al., 1989).

The pharmacokinetics of the standard activators, given by bolus dosing, were compared in guinea pigs (fig. 4) using methods described previously (Nunn et al., 1987). SK-plasminogen, t-PA, scu-PA, and tcu-PA were all cleared rapidly (half-life <2 min), whereas the clearance of APSAC was slow (half-life approximately 90 min) and largely regulated by the rate of deacylation

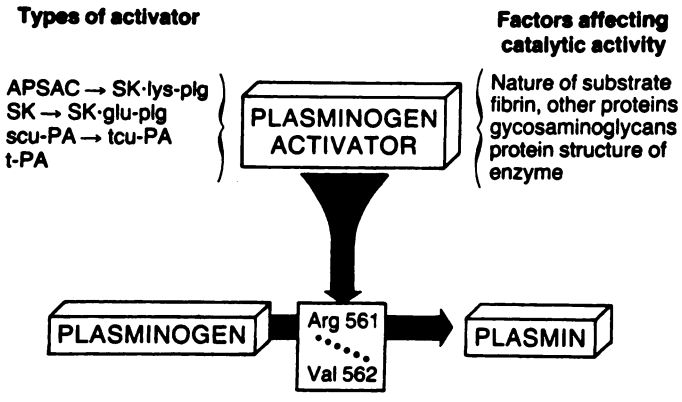


FIG. 2. Biochemical mechanism of action of standard thrombolytic agents. plg, plasminogen.

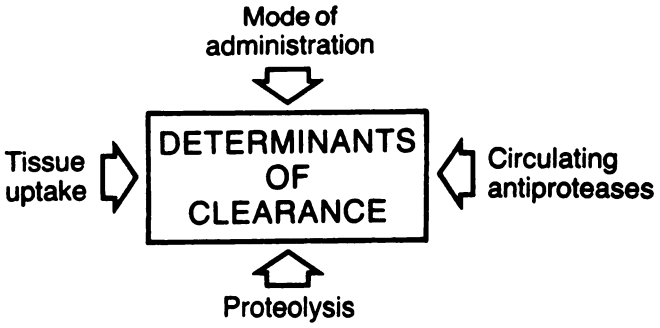


FIG. 3. Major influences on circulating activity of thrombolytic agents.

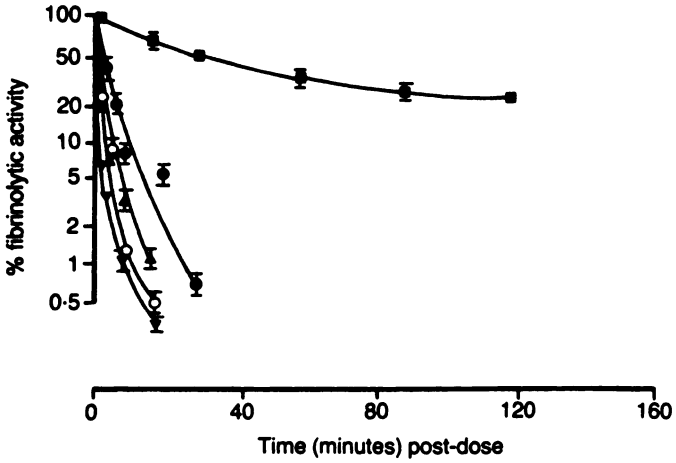


FIG. 4. Plasma clearance of fibrinolytic activity in guinea pigs. All activators were given by bolus intravenous injection: t-PA, 0.32 nmol/kg body weight (Δ); scu-PA, 5 nmol/kg (●); tcu-PA, 7.4 nmol/kg (▼); SK-plasminogen, 0.53 nmol/kg (○); APSAC, 0.53 nmol/kg (■). Experimental details are described in Nunn et al., 1987.

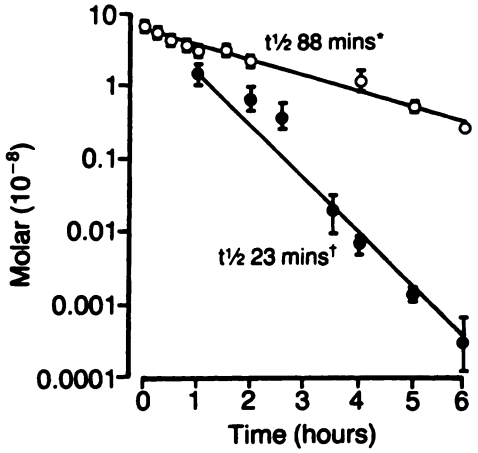


FIG. 5. Pharmacokinetics of SK and APSAC in patients with AMI. Results from the original publications on SK (●) (n = 7, Mentzer et al., 1986†) and APSAC (○) (n = 6, Been et al., 1986*) are expressed on a common basis of molarity.

(Hibbs et al., 1989). The rapid clearance of scu-PA, tcu-PA, and t-PA in guinea pigs (and rabbits, Nunn et al., 1987) confirms other findings in experimental animals and in patients with AMI (for examples, see Bounameaux et al., 1986; Stump et al., 1987; Verstraete, 1989). The slow clearance of APSAC relative to SK has been confirmed in patients with AMI (Been et al., 1986: Mentzer et al., 1986; Köhler et al, 1987; Fears et al., 1990b). For example, representative studies of SK and APSAC are compared in fig. 5 using a common basis (plasma drug level in molar units). SK was cleared significantly faster than APSAC (half-life 23 vs. 88 min). In addition, its peak level was lower and was achieved later after initiation of therapy. Because of its long half-life in the circulation, APSAC can be given as a single, intravenous injection (during 2–5 min). This is in contrast with the lengthy intravenous infusions required to maintain blood levels of the other thrombolytic agents (1–6 h). Furthermore, as a proenzyme, APSAC can be administered rapidly without incurring an unacceptable hypotensive response that can accompany the rapid dosing with SK; the latter effect may be the result of an initial, fast plasmin generation and kinin production (Green et al., 1984).

Because u-PA and t-PA are principally removed from the circulation by hepatic uptake, circulating levels of those substances will be influenced by changes in liver function (Bounameaux et al., 1986; Brower et al., 1988) and, hence, response to a standardised dosage regimen may be affected by hepatic status.

The clearances of SK and APSAC may be less dependent on variations in hepatic function because the circulating activity of SK-plasminogen declines as a result of proteolysis in the blood stream of the SK moiety in the activator complex to lower molecular weight, inactive fragments (Standring, et al., 1988) and because of inhibitor complex formation in the blood stream. Whether or not specific inhibitors of SK-plasminogen exist has been a matter of controversy (Verstraete and Collen, 1986), but evidence for neutralisation by human $\alpha_2$-antiplasmin (Standring et al., 1988) has now been confirmed (Gonias et al., 1988). The long circulating residence time of APSAC reflects the protective effect of acylation on the stability of the activator complex; loss of activity is dependent on deacylation (Standring et al., 1988; Fears, 1989c).

*2. Reaction with PAI-1.* It is also possible that the thrombolytic response to t-PA and tcu-PA can be influenced by variations in level of endogenous antiproteases, particularly PAI-1, and other circulating proteins. Variations in the level of PAI-1 may act to inhibit endogenous fibrinolysis and, hence, predispose to thrombosis (Hamsten et al., 1985). Some patients may have a circulating level of PAI-1 sufficient to inhibit exogenous t-PA (Brommer et al., 1985). Although plasma levels of PAI-1 are usually low relative to the plasma levels of t-PA and tcu-PA reached after drug administration, a high concentration of PAI-1 (which can bind to fibrin) may be achieved in the vicinity of the clot by release from the $\alpha$ granules of activated platelets (Wagner et al., 1989) or from a reservoir located in the extracellular matrix (Gerard and Meidell, 1989). It has been suggested that a circadian variation in clinical response to t-PA might be related to a circadian variation in PAI-1 activity (Becker et al., 1988). Moreover, a rapid increase in plasma PAI-1 levels, induced in response to t-PA administration, may neutralise endogenous fibrinolytic activity and, hence, be of importance in determining the incidence of reocclusion (Lucore and Sobel, 1988).

*3. Potential inhibitory role of other circulating proteins.* Apart from the protease inhibitors, other circulating proteins may, theoretically, impair the activity of plasminogen activators. The lipoprotein subfraction, Lp(a), shows sequence homology to plasminogen and has been

implicated in both atherogenesis (inducing foam cell formation) and thrombosis (Scott, 1989; Miles et al., 1989) (fig. 6). Lp(a) can act as a potent competitive inhibitor of SK (Edelberg et al., 1989) and, by binding to fibrin and displacing plasminogen, may inhibit t-PA (Brandstrom et al., 1988; Loscalzo et al., 1988). On this theoretical basis, APSAC and tcu-PA, which activate systemic plasminogen (and, therefore, may be less dependent than t-PA on fibrin-bound plasminogen as substrate) and would not be expected to be subject to competitive inhibition, are less likely to be influenced by Lp(a).

Varying levels of anti-SK antibodies are present in patients as a result of previous streptococcal infections and these antibodies bind SK. The relationship between antibody binding and SK inactivation has been unclear, but studies in model systems in vitro suggest that the inhibition of fibrinolysis by SK or APSAC is small at the normal levels of antibody (anti-SK IgG) seen in a population (Fears et al., 1987; Fears, 1989c). High levels of anti-SK IgG do not affect the deacylation rate of APSAC (Hibbs et al., 1989) and antibody is unlikely to be a major factor in the clearance of APSAC in most patients (Been et al., 1986; Fears et al., 1990b). In a clinical comparison of intravenous APSAC and intracoronary SK, little influence of antibody on clinical end points was noted (Hoffmann et al., 1988).

### *D. Thrombus Affinity and Selectivity*

*1. Characterisation of fibrin binding.* Methods of measurement for quantifying fibrin binding and the structural characteristics of those plasminogen activators that bind specifically to fibrin have been reviewed previously (Fears, 1989a). In model conditions in vitro t-PA and APSAC demonstrate similar high affinity for fibrin, whereas scu-PA and tcu-PA do not bind to fibrin.

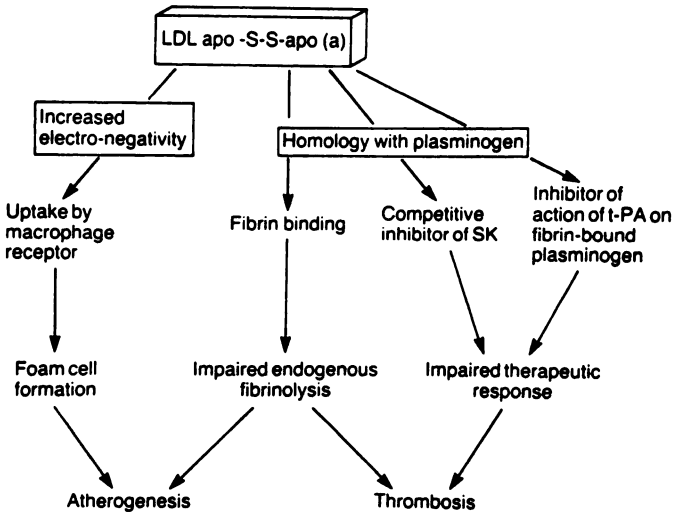


FIG. 6. Potential function of Lp(a) [low-density lipoprotein (LDL)-apoprotein (apo)(a)] in atherogenesis and thrombosis and in the response to t-PA and SK.

The binding of t-PA is mediated by the A-chain (Dodd et al., 1986). Recent research (de Munk et al., 1989) has confirmed the initial evidence suggesting the involvement of specific domains in the A-chain, the second Kringle domain, and the fibronectin-like finger region (reviewed by Fears, 1989a). Extra binding sites for t-PA are uncovered after the initial proteolysis of fibrin, although factor XIIIa-catalysed cross-linking of fibrin may mask high-affinity sites (Husain et al., 1989).

The binding of SK-plasminogen and APSAC to fibrin is mediated by the Kringle domains on the A-chain of plasmin(ogen), but the formation of an activator complex with SK imparts additional fibrin affinity to plasminogen (Cederholm-Williams, 1981). Thus, SK-activator complexes, whether or not stabilised as the acylated proenzyme, are bound to clot fibrin in model systems (fig. 7, experiment i) to a significantly greater extent than is the parent zymogen. Furthermore, the greater binding originally observed for Lys-78-plasminogen substrate compared to Glu-1-plasminogen (reviewed by Fears, 1989a) is accentuated in greater binding of those activator complexes containing Lys-plasminogen compared to those containing Glu-plasminogen. With the conditions of experiment i presented in fig. 7 there is no difference between the binding of anisoylated complexes and the corresponding free activator complex, but uptake was measured in the presence of aprotinin (to stabilise the clot matrix) and the "free" activator will form an active site-inhibited complex with aprotinin. When uptake into the clot is measured in the presence of soybean trypsin inhibitor (which does not bind to SK-plasminogen), when the free activator can mature to SK-plasmin (Fears et al., 1989c), the binding of the anisoylated, stabilised, activator was significantly greater (fig. 7, experiment ii). These results may have clinical relevance because the complex initially formed after administration of SK (SK-Glu-plasminogen) is likely to bind to the thrombus less well than APSAC. The higher clot affinities of activators containing Lys- rather than Glu-plasminogen are sensitive to interference by EACA (Fears et al., 1989c). Thus,

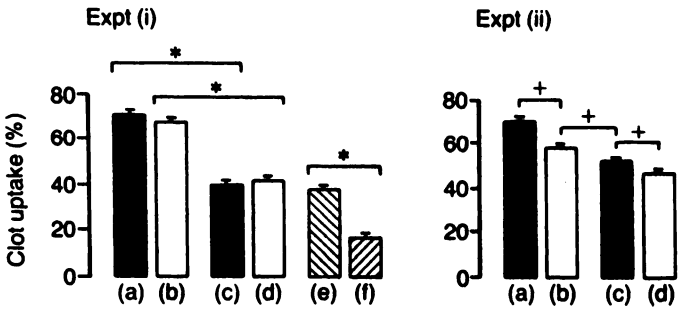


FIG. 7. Measurement of clot binding in vitro. Uptake of $^{125}$I-protein ($4.9 \times 10^{-8}$ M into forming human plasma clots (Fears et al., 1989c) was measured in the presence of 10 $\mu$M aprotinin (experiment i) or 2 $\mu$M soybean trypsin inhibitor (experiment ii): a, APSAC; b, SK-Lys-plasminogen; c, Glu-plasminogen variant of anisoylated activator complex; d, SK-Glu-plasminogen; e, Lys-plasminogen; f, Glu-plasminogen. Results are means ± SEM for six replicates. *$P < 0.001$, †$P < 0.01$.

from a clinical perspective, whatever is gained by the higher fibrin affinity of APSAC over the complex formed by SK, it is easily reversed by EACA. EACA (and related lysine analogues) remains as effective an antidote to APSAC as to SK if it is desired to arrest the pharmacological action. The fibrin binding of t-PA cannot be entirely reversed by EACA (de Munk et al., 1989, Husain et al., 1989); that is, the binding mediated by domains other than the Kringle domain is not impaired by EACA.

*2. Consequences of fibrin binding.* Binding of a plasminogen activator to fibrin creates several potential advantages in thrombolytic therapy. Targeting of an agent with fibrin affinity to the thrombus should increase potency, particularly if a high plasma/thrombus gradient in drug level can be achieved initially by rapid administration. Not only does fibrin binding lead to an accumulation of agent in the thrombus but colocalisation of activator and plasminogen substrate on the fibrin template also can promote the efficiency of enzymatic activity. Fibrin enhancement of catalytic efficiency was first demonstrated for t-PA (Hoylaerts et al., 1982) and, subsequently, for SK-Lys-plasminogen (Fears et al., 1985b), both activators acting by the same catalytic mechanism: rapid equilibrium-ordered bireactant sequence (Cassels et al., 1987). The relative fibrin enhancement in model conditions (table 4) is greater for t-PA than for SK-Lys-plasminogen, but the absolute enzymatic activity in the presence of fibrin is similar, and SK-Lys-plasminogen (and, hence, APSAC) binds to fibrin at least as tightly as does t-PA (table 4). The major difference is the catalytic activity in the absence of fibrin as cofactor. SK-plasminogen is much more active and, thus, at clinical doses will induce greater systemic activation and fibrinogen depletion. tcu-PA also demonstrates a high basal activity but does not specifically bind to fibrin and is not enhanced in activity by fibrin. It is important to appreciate, therefore, that fibrin selectivity is related to basal activity and the degree of fibrin enhancement rather than to degree of fibrin affinity.

One other consequence of high fibrin affinity is the prolongation of clot lytic activity even when systemic levels of activator are low. Sustained fibrinolysis, in vitro, after brief exposure of clot to activator was first demonstrated for APSAC (Fears et al., 1985a) and, subsequently, for t-PA (Baldus et al., 1988). The continuing lysis can be attributed to fibrin binding because no activity was obtained for tcu-PA, and activity was significantly greater for APSAC than the Glu-plasminogen homologue (fig. 8). Sustained efficacy of this type may be clinically important even for an agent with a long circulating residence time: there is preliminary evidence in patients with AMI (Brochier, 1987) that thrombus dissolution and increase in lumen size can continue for some hours after dosing with APSAC.

*3. Systemic lytic state.* When one considers the systemic changes occurring after thrombolytic therapy, two points should be appreciated. First, all clottable protein measured in standard assays may not represent intact fibrinogen with normal haemostatic function (Marder, 1989). Second, interpretation of differences in response to the standard agents is complicated by the potential for laboratory artifacts whereby proteolysis continues during sample handling unless an exogenous inhibitor is added to the sample. Although many investigators have used inhibitors such as aprotinin to curtail plasminogen activation and plasmin action, the degree of protection achieved may have been inadequate (Fears et al., 1989b) and early fibrinogenolysis in patients may often be overestimated. Nonetheless, at the usual clinical doses, SK, tcu-PA, and APSAC produce more fibrinogenolysis than does t-PA although, as discussed in the preceding section, the degree of systemic activation is not inversely correlated with fibrin affinity per se.

It has become clear that biochemical selectivity (that is, relatively greater lysis of fibrin than fibrinogen as exemplified by t-PA) does not diminish the risk of bleeding. Although fibrinogen depletion (systemic lytic state) may contribute to a haemostatic defect, in practice haemostasis may be influenced more by adjunct therapies such as aspirin and heparin (Timmis et al., 1986). Fur-

TABLE 4

*Fibrin affinity and catalytic efficiency of Lys-plasminogen activation*

| Plasminogen activator | Dissociation constant, soluble fibrin ($K_D$, nM)* | Second-order rate constant† ($k_{cat}/K_m$ $\mu M^{-1}$ $sec^{-1}$): | |
|---|---|---|---|
| | | Fibrin | Cofactor |
| | | − | + |
| t-PA | 60 | 0.12 | 52 |
| tcu-PA | 1300 | 3.3 | 2.9 |
| SK-Lys-plasminogen | 15 | 12 | 68 |

* Cassels et al., 1987; Lijnen et al., 1986.

† Fears et al., 1985b; Cassels et al., 1987; Fears, 1989.

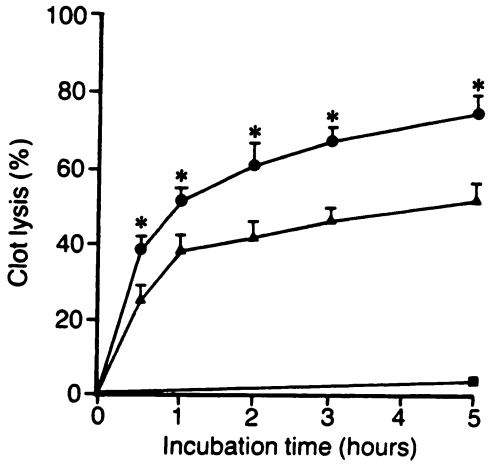


FIG. 8. Sustained fibrinolysis in vitro. Clot lysis was measured on return to buffer after 1 min exposure to thrombolytic agent in plasma (Fears et al., 1985a). Human plasma clots (radiolabeled with $^{125}$I-fibrinogen) were incubated with APSAC (●) ($2 \times 10^{-6}$ M) or the Glu-plasminogen homologue (△) ($2 \times 10^{-6}$ M) or tcu-PA (■) ($2.5 \times 10^{-6}$ M) for 1 min. Results are means ± SEM for five replicates. *$P < 0.05$.

thermore, fibrin selectivity cannot be equated to thrombus specificity because t-PA, like the other activators, will induce fibrin digestion both in the thrombus and in the haemostatic plug. It is the lysis of this wound fibrin that leads to bleeding (Timmis et al., 1984) and, in direct clinical comparisons, the incidence of haemorrhage after t-PA therapy is no less than that occurring after SK (Rao et al., 1988). Because t-PA binds to endothelial cells with a subsequent enhancement of plasminogen activation (reviewed by Fears, 1989a), the possibility also exists of aggravating local vessel damage and bleeding. However, the suggestion that the use of an agent such as t-PA may be associated with a greater risk of severe bleeding, in particular intracranial haemorrhage (Marder, 1989), requires further proof.

There may be beneficial consequences of limited fibrinogen depletion that augment the response to thrombolysis. The reduction in circulating fibrinogen levels removes substrate for rethrombosis, and it is remarkable that the original enthusiasm for t-PA as a fibrin-selective agent is being tempered by an acceptance of the merits of combination therapy with tcu-PA (Califf et al., 1989). The rationale for coadministering these activators is the potential combination of the benefits of high fibrin affinity (t-PA), achieving initial patency, and systemic activation by tcu-PA conferring protection from reocclusion. This combination of pharmacological attributes can be found in a single agent such as APSAC (see preceding section).

Reduction in plasma fibrinogen also leads to a decrease in blood viscosity and, because high blood viscosity may delay delivery of t-PA to the thrombus (Brower et al., 1988), those thrombolytic agents that lower blood viscosity may have improved access to the occlusion. Furthermore, reduced blood viscosity could be associated with improved coronary microcirculation and decreased workload on the heart (Chamberlain, 1989). In a direct clinical comparison, SK decreased viscosity in both plasma and blood, whereas t-PA did not (Jan et al., 1985), and it was suggested that, for a given degree of arterial patency, myocardial blood flow may be better maintained with SK than t-PA.

There is controversy as to whether thrombolytic agents achieve therapeutic benefit essentially only by dissolution of thrombi or whether other pharmacological effects (including changes in blood viscosity) may be important (Chamberlain, 1989). Although a mechanism was not elucidated, it has been found in a dog model of coronary thrombosis that SK can induce some myocardial salvage, regardless of whether reperfusion is established (Kopia et al., 1988). Initial studies of t-PA in cats (Darius et al., 1986) also suggested a cardioprotective effect independent of thrombolysis, but later results in cat and dog models (Kloner et al., 1989) could not confirm the benefit.

*4. Potential for hypercoagulability.* In addition to its effect on fibrinogen, systemic plasmin can act on other components of the coagulation cascade. The initial proteolysis of factor V produces fragments with procoagulant activity (Lee and Mann, 1989). Both t-PA and SK can induce thrombin activity in vitro (Eisenberg and Miletich, 1989) and in patients (Owen et al., 1988) but, because of the concomitant depletion of fibrinogen, the effect of SK is likely to be associated with less new thrombus formation. Such biochemical changes may help to explain the relatively high reocclusion rate seen for t-PA in some clinical trials (see section V, B, 2). Furthermore, plasminogen activation at the endothelium in response to t-PA inhibits prostacyclin biosynthesis (Schafer et al., 1989) and may predispose locally to thrombosis.

Thrombin is not necessarily susceptible to inactivation by heparin-antithrombin III because of protective sequestration by fibrin (Hogg and Jackson, 1989). Heparin action also may be impaired by local platelet activation (increasing the conversion of prothrombin to thrombin and neutralising heparin with platelet factor 4 and thrombospondin). Synthetic thrombin inhibitors such as argatroban may be more efficacious in enhancing the activity of t-PA, at least experimentally (Fitzgerald and Fitzgerald 1989).

The clinical need for adjunctive therapy with heparin along with t-PA is currently being examined (see section V, B, 6). It is important to appreciate that heparin can have deleterious actions that may tend to offset any beneficial influence on coagulation. For example, heparin can increase circulating fatty acid levels as a consequence of activating lipoprotein lipase (Magnani, 1989) with a potential for inducing ventricular arrhythmias (Riemersma et al., 1982).

## V. Clinical Efficacy

### *A. History of Acute Myocardial Infarction*

Observations relevant to the perception of coronary occlusion as the cause of ischaemic heart disease and to AMI as a clinical entity also can be traced into antiquity. The earliest records were probably those of the Egyptians who described precordial pain and seemed to be aware of the notion of obstruction. Following the delineation by Galen of the nutrient function of the coronary vessels, there was a long decline in medical knowledge until the Renaissance brought further revelations in anatomy and physiology. Subsequently, Hoffmann, in 1738, expressly related changes in the viscosity of blood to coronary obstruction and Morgagni, in 1761, explained myocardial pathology as a consequence of coronary occlusion. Some of the ensuing discoveries that form the basis of the modern concept of AMI as a specific disorder are listed in table 5.

Although coronary heart disease has probably contributed to mortality and morbidity at least since the time of the development of the first city states (Leibowitz,

TABLE 5
*Key events in the recognition and treatment of AMI*

| Year | Investigator | Achievement |
|---|---|---|
| 1778 | Jenner | Description of coronary occlusion |
| 1782 | Heberden | Definition of angina |
| 1878 | Hammer | Diagnosis of coronary thrombosis during life |
| 1910<br>1912 | Obrastzow and Straschesko<br>Herrick | Demonstration that coronary thrombosis is a discrete, diagnosable, clinical state |
| 1919 | Herrick | Electrocardiographic tracing from patient with AMI |
| 1954 | La Due and Wroblewski | Recognition of circulating enzyme changes as monitor for AMI |
| 1959 | Sherry et al. | Clinical use of thrombolytic agent |
| 1960 | Boucek and Murphy | Selective delivery of thrombolytic agent to coronary arteries |
| 1962 | Sones and Shirey | Introduction of selective coronary angiography |
| 1980 | De Wood et al. | Proof that coronary occlusion is responsible for >80% AMI |

1970), it is usually considered as a 20th century epidemic. Mortality from cardiovascular disease in men accounts for approximately half of mortality from all causes in most European countries; for women it contributes 30–40% to the total (Smith and Tunstall-Pedoe, 1984). In the United Kingdom, approximately 160,000 people die each year as a consequence of AMI and ischaemic sudden death.

### *B. Clinical End Points*

*1. Methods of evaluation.* The immediate objective of thrombolytic therapy for AMI is to lyse the thrombus in the infarct-related artery and to restore blood flow to the ischaemic myocardium. Timely renewal of oxygen and nutrients should lead to diminution in the ultimate size of the infarct, recovery of left ventricular function, and an improvement in morbidity and survival (table 6).

Recanalisation of the vessel is assessed definitively by coronary angiography performed prior to therapy to confirm diagnosis and after therapy to establish whether flow has been restored. The approximate time to reperfusion can be estimated by serial angiograms. Patency studies differ in that pretreatment angiograms are omitted and occlusion is inferred from clinical signs or electrocardiographic evidence. Patency rates are often 10–20% higher than reperfusion rates because the artery may not initially be occluded by a thrombus in a proportion of patients and because efficacy may be improved by the earlier intervention that is possible when the pretreatment angiogram is omitted.

*2. Restoration of flow.* Data for the standard agents administered intravenously are summarised in table 7. Apart from variability in biochemical factors (section IV, C), drug effectiveness may be influenced by adjunct therapies and by the location, size, and age of the thrombus. It is difficult to summarise the literature regarding t-PA and scu-PA because of variations in regimen (size

TABLE 6
*Determination of clinical response to thrombolytic agents in AMI*

| End point | Principal assessments |
|---|---|
| Vessel recanalization | Invasive: angiography<br>Noninvasive: clinical signs, enzyme release |
| ↓ Infarct size | Scintigraphy, enzyme release, nuclear magnetic resonance imaging, electrocardiogram evolution |
| ↑ Left ventricular function | Contrast or radionuclide ventriculography: regional and global wall motion |
| ↓ Coronary morbidity and mortality | |

TABLE 7

*Effect of thrombolytic agents on recanalization of coronary arteries (treatment within 6 h of onset of symptoms)*

| | SK* | t-PA* | APSAC† | tcu-PA‡ | scu-PA§ |
|---|---|---|---|---|---|
| Dose regimen | $1.5 \times 10^6$ IU in 1 h | 80–150 mg (1 chain/ 2 chain in ≤6 h | 30 U in 2–5 min | $3 \times 10^6$ IU in 90 min | ≤80 mg in 60–90 min |
| Early angiographic end point (approx. 90 min): | | | | | |
| Reperfusion (%) | 30–60 | 60–75 | 50–70 | ¶ | 50 |
| Patency (%) | 45–75 | 60–90 | 70–100 | 66 | 60–90 |
| Average time to reflow (min) | 45 | 45 | 45 | ¶ | ¶ |
| Reocclusion at 24 h (%) | 4–20 | 4–20 | 4 | ¶ | ¶ |

* Reviewed by Collen et al. (1989).
† Reviewed by Anderson (1989); Cregeen and Duff (1989).
‡ Neuhaus et al. (1988).
§ Verstraete and Vaughan (1989); Loscalzo et al (1989).
¶ Insufficient data.

of dose, duration of infusion) and because of possible pharmacological variation between different pharmaceutical preparations (one-chain and two-chain variants of t-PA, natural and recombinant scu-PA). Most of the published data concerning t-PA does not refer to the current standard regimen (100 mg, predominantly one-chain obtained from a suspension culture of Chinese hamster ovary cells, delivered as a 10-mg bolus followed by hourly infusions of 50, 20, and 20 mg). One study of this dose in conjunction with intravenous heparin and aspirin (Simoons et al., 1988) suggests a patency rate of 68%. Dosage regimens for scu-PA have yet to be standardised and there is controversy regarding the need for a complementary priming dose of tcu-PA (Gulba et al., 1989; Loscalzo et al., 1989).

In direct comparisons of agents, there is evidence that t-PA can achieve greater early recanalisation than can SK (reviewed by Collen et al., 1989) and that APSAC achieves an efficacy similar to the intracoronary delivery of SK (the original clinical approach to maximising efficacy) but greater than the standard intravenous administration of SK (reviewed by Anderson, 1989). Other investigators have found that the efficacy of t-PA was similar to tcu-PA (Neuhaus et al., 1988) and scu-PA acted faster than SK (PRIMI Trial Study Group, 1989).

At present, there is controversy regarding whether or not the measurement of early vessel opening is a useful end point. Because benefit will only be obtained if patency is maintained, reocclusion rates must be taken into account. Early reocclusion can occur because the initial stimulus provided by damage to the vessel wall has not been repaired. Reocclusion may, theoretically, be reduced by adjunct anticoagulant or antiplatelet therapies, by depleting circulating fibrinogen, and by maintaining circulating levels of plasminogen activator to lyse any new formed fibrin. The very high reocclusion rates that were seen in early trials of t-PA (for example, Gold et al., 1986) may not occur with current dosage regimens, although total in-hospital morbidity and mortality rates (angiographically defined reocclusion plus reinfarction plus death) have remained high in some pivotal trials. For example, in the TIMI-1 trial (Grossbard, 1988), such rates (reocclusion, reinfarction, and death) totaled 29% for t-PA and 30% for SK. Reocclusion rates are influenced by the frequency of surgical interventions, such as percutaneous transluminal angioplasty, and data are particularly scarce for tcu-PA and scu-PA. Although there have been no large, direct, comparisons of agents, reocclusion rates seem lowest for APSAC, 4% as determined by repeat angiography at 24 h in the absence of aspirin therapy (Relik-Van Wely et al., 1988). From the pooled data base (3850 patients) on APSAC (Cregeen and Duff, 1989) the recurrence rate within 3 days (angiographically defined reocclusion and clinically defined reinfarction) was 4%, and, from a smaller data base (Anderson, 1989), reinfarction at any time during convalescent observation totaled 8.5%.

One other reason why early patency may not be a sufficient end point is because slower restoration of flow may also be beneficial. Late patency has been suggested as important (Chamberlain, 1989) in infarct healing, as a conduit for sustaining collateral flow, and as a method to reduce the incidence of late potentials and, hence, the likelihood of sudden death (arrhythmia).

Only APSAC is administered as a single, intravenous injection (2–5 min). The other agents are routinely given as intravenous infusions ranging from 60 min (SK) to 3 h (t-PA) (table 7). APSAC can be given in this manner because of slow plasma clearance rates (see section IV, C), and the convenience of administration facilitates the early treatment of the maximum number of patients and may lead to the extension of treatment to pre-hospital use (Castaigne et al., 1989). There is limited experience with the delivery of the other thrombolytic agents by single intravenous injection. Although the initial results for tcu-PA were encouraging (Mathey et al., 1985), current usage is to combine an initial bolus with infusion up to 90 min (Neuhaus et al., 1988). Rapid administration of high doses of tcu-PA may lead to haemodynamic instability (Dickie et al., 1974) as it does for SK (Lew et al., 1985; Hall, 1987). Bolus administration of SK also appears to be associated with an early high reocclusion

rate, at least in a small group of patients (Wenzel et al., 1987), and this is consistent with the short half-life of SK. Initial studies of the use of bolus t-PA are also appearing (Tebbe et al., 1989), but relatively high rates of reocclusion (22% by 24 h) and recurrent ischaemic events were observed despite the use of percutaneous transluminal angioplasty.

*3. Myocardial salvage.* Analysis of the degree of salvage of jeopardised myocardium has commonly been made by following changes in ventricular function (table 6). Many factors operate to confuse the interpretation of such measurements. For example, baseline function may be abnormally high because of compensatory hypercontractility (catecholamine drive), the myocardium may be stunned at an early assessment time, and global function measurements may be confounded by hyperkinesia of healthy segments. Nonetheless, various studies of the effects of thrombolytic drugs on ventricular function have been attempted (reviewed by Rappaport, 1989; Topol and Califf, 1989; Van de Werf, 1989), and the major placebo-controlled, randomised trials are summarised in table 8.

The effects of the thrombolytic agents on ventricular functions appear broadly comparable, however the responses to thrombolytic therapy will be influenced by factors that may vary among trials. These variables include infarct site location (inferior or anterior), time from onset of symptoms to treatment, time of assessment, first or recurrent infarct, and frequency of surgical intervention. Results also may differ according to technique used, as exemplified by the TICO study in which t-PA was used (O'Rourke et al., 1988), and the improvement in left ventricular ejection fraction achieved statistical significance by using contrast ventriculography but not by radionuclide ventriculography.

Smaller studies of t-PA (Armstrong et al., 1989; Bates et al., 1989) and APSAC (Been et al., 1987; Buchalter et al., 1987) have confirmed at least some benefit on function. In one direct comparison (White et al., 1989), the effects of SK and t-PA on ejection fraction and end-systolic volume were similar. In an unblinded and smaller study (Magnani, 1989), the improvement in ejection fraction was greater after t-PA than SK at the time of hospital discharge but not at 4 days, and left ventricular index score (summing effects on regional wall motion) was equivalent for the two agents. Although not a randomised comparison, one group of workers evaluated both SK (Bassand et al., 1987) and APSAC (Bassand et al., 1989); there was some evidence for a greater effect for APSAC, i.e., an improvement in function in both anterior and inferior infarct patients.

Fewer investigators have endeavoured to assess infarct size directly, and there is no consensus regarding methods of measurement: enzyme changes, nuclear magnetic resonance, and scintigraphy are all used. Using nuclear magnetic resonance, Wisenberg and coworkers (1988) found a significant reduction in infarct size after SK therapy. When assessed by $Tl^{201}$ scintigraphy, APSAC significantly decreased infarct size (Bassand et al., 1989) but, when Tl scintigraphy was used in conjunction with an exercise test, there was no statistically significant reduction in infarct size after t-PA (Armstrong et al., 1989).

*4. Survival.* The ultimate goal of thrombolytic therapy is to improve long-term survival. Several large, controlled studies of SK, t-PA, and APSAC have demonstrated improved survival, usually at about 30 days after treatment (fig. 9). The changes all achieved statistical significance except in the ECSG trial of t-PA, which had not been designed as a mortality study.

Both the control mortality rate and the response to therapy may be influenced by variables that differ among the studies. These factors include the delay occurring between onset of symptoms and treatment; the results in fig. 9 are compared only for those patients recruited within 6 h. Patients treated later may benefit less, although evidence from the largest trial (ISIS-2) indicates that some response can be obtained even when SK is administered 12–24 h after onset of symptoms. Whatever the mechanism for this late benefit, there is a consensus that the best response is achieved by the earliest intervention. One other major variable is the nature of the

TABLE 8

*Effect of thrombolytic agents on left ventricular function (treatment within 6 h of onset of symptoms): Randomised studies*

| | SK* | t-PA† | APSAC‡ |
|---|---|---|---|
| Left ventricular ejection fraction (%) | | | |
| Control (placebo/heparin) | 44–54 | 46–54 | 47 |
| Thrombolytic agent | 45–49 | 51–61 | 53 |
| % Change from control | 2–11 | 5–15 | 13 |

* Bassand et al. (1987); Topol and Califf (1989; total, 4 studies).

† Topol and Califf (1989; total, 5 studies).

‡ Bassand et al. (1989).

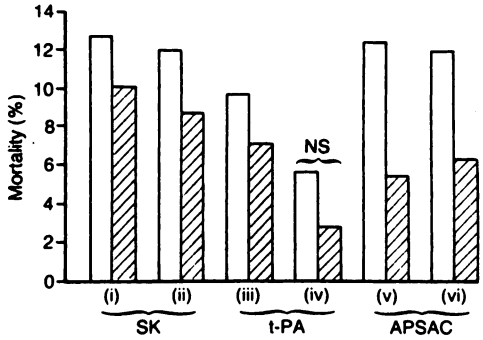


FIG. 9. Major randomised, placebo-controlled clinical trials measuring mortality after thrombolytic therapy. Results (total or cardiovascular mortality) are presented for patients with AMI recruited within 6 h of onset of pain and assigned to a control group (white bars) or thrombolytic therapy (hatched bars): i, GISSI (GISSI, 1986), n = 9743, measured at 21 days after dosing; ii, ISIS-2 (ISIS-2 Collaborative Group, 1988), n = 10,710, 5 weeks; iii, ASSET (Wilcox et al., 1988), n = 5011, 1 month; iv, ECSG (Van de Werf and Arnold, 1988), n = 721, 14 days; v, GEMT (Meinertz et al., 1988), n = 313, 28 days; vi, AIMS (AIMS Trial Study Group, 1988), n = 1251, 28 days.

diagnostic criterion for recruitment, that is, stringent evidence for electrocardiographic changes (trials i and iv–vi in fig. 9) or only clinical symptoms (trials ii and iii). For example, the low rates in both control and t-PA-treated groups in the ASSET study (trial iii), 9.8 and 7.2%, respectively, are increased to 11.2 and 8.5%, respectively, if only those patients are analysed who had an abnormal electrocardiogram at the time of admission (Wilcox et al., 1988). Survival also can be influenced by adjunct therapy. In particular, it has been shown that aspirin provides benefits additional to SK (ISIS-2 study), although the mechanism of the effect is not yet certain. Use of adjunct aspirin may also have contributed to the low mortality rates seen for both the control and t-PA-treated groups in the ECSG study (iv in fig. 9); aspirin use was low in the other trials compared in fig. 9.

There are fewer data published concerning long-term survival. Net reduction in mortality at 1 year after SK therapy (GISSI 1987) for those treated within 6 h was 12%, compared to 20% reduction at 21 days (fig. 9). For APSAC, the net reduction in mortality observed for the first 1004 patients at 30 days (48%) has been maintained in preliminary estimates of 1 year mortality (44%) (Julian, 1989).

Because of the various possible confounding influences and because there have not been direct comparisons of thrombolytic agents, it is not yet possible to conclude with certainty that there are differences between the individual agents with regard to long-term survival. However, the biggest individual effect has been seen for APSAC (fig. 9), whether calculated in terms of percentage reduction from control or in terms of number of lives potentially saved per 100 patients treated. This conclusion is reinforced by the pooled analysis made by Yusuf and coworkers (1988) of all reported trials (fig. 10). The results of the direct comparisons currently being conducted (GISSI-2 and ISIS-3 trials) are eagerly awaited and will be of considerable interest with regard to choosing the optimum therapy. Other key questions, such as patient selection criteria (Chamberlain, 1989), also may be, at least partly, answered by the trials in progress.

*5. Safety and tolerance.* Space does not permit a detailed review but, broadly, at current therapeutic doses, t-PA, SK, and APSAC appear similar with regard to incidence of adverse events (there is insufficient experience with tcu-PA and scu-PA). Although SK and APSAC are antigenic, the incidence of allergic reactions is low (ISIS-2 and AIMS data base). The incidence of allergic events that has been seen for t-PA (Linnik et al., 1989) probably reflects the uncertain nature of the clinical diagnosis.

The most common side effect is bleeding. The reported incidence is influenced by anticoagulant and antiplatelet adjunct therapies, by the nature of invasive procedures, and for minor events by the assiduity with which the patient is monitored. In a review of the results for SK in

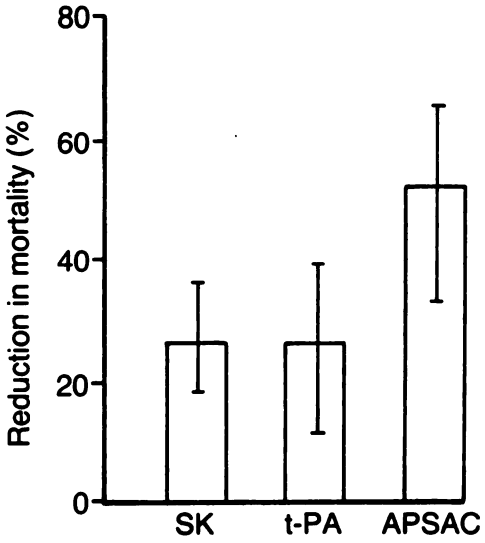


FIG. 10. Overview of mortality trials (Yusûf et al., 1985, 1988). Results are compared for those patients with AMI recruited within 6 h of onset of pain and expressed relative to control rates in randomised trials. Changes are expressed as the mean with 95% confidence intervals.

15,000 patients from seven major intracoronary or intravenous trials (Fennerty et al., 1989), the incidence of major haemorrhage was 1%, central nervous system bleeding was 0.15%, and fatal bleeding was 0.04%. As expected, the frequency of major haemorrhage was much greater in invasive (15%) than noninvasive (0.8%) studies, the excess being associated principally with coronary artery catheterisation. In a review of 2000 patients receiving t-PA (Fennerty et al., 1989), most of whom received invasive procedures, major haemorrhage was 12%, central nervous system bleeding, 0.6%, and fatal bleeding, 0.1%. In direct comparisons of SK and t-PA (for example, Rao et al., 1988) there was no difference in major bleeding. In a review of data from nearly 4000 patients receiving APSAC (Cregeen and Duff, 1989), severe haemorrhagic events totaled 1.9% (vs. 0.4% in control patients). The incidence of cerebrovascular accidents, regardless of aetiology (not all haemorrhagic), within 7 days was 0.8% (vs. 0.3% in control patients). For scu-PA the initial, very limited, information from an invasive study suggests that bleeding could be less common than for SK (PRIMI Trial Study Group, 1989). Bleeding after tcu-PA appears approximately equivalent to t-PA (Neuhaus et al., 1988).

*6. Adjunct therapies.* Apart from the major issues of which thrombolytic agent to choose, when to use, and whom to treat, a key remaining question is what adjunct therapies should be used (Chamberlain, 1989). Some examples are listed in table 9. Antiplatelet agents, at least, are likely to become a standard accompaniment. Novel antiplatelet agents may be potent and selective with regard to preventing thromboxane formation and able to demonstrate good activity both in experimental animals (Fitzgerald and Fitzgerald, 1989) and in patients

TABLE 9
*Examples of potential adjunct therapies in thrombolysis*

| Agent | Possible benefit |
|---|---|
| Antiplatelet agents | ↓ Reocclusion |
| Anticoagulants | ↓ Reocclusion |
| Nitrates, prostaglandin $E_1$ | ↓ Oxygen demand, ↑ collateral flow, ↓ platelet aggregation, ↑ access (vasodilation) |
| $Ca^{2+}$ antagonists, β-blockers | Various (reviewed by Chamberlain, 1989; Opie, 1989; Verstraete and Vaughan, 1989) |
| Angiotensin-converting enzyme inhibitors | Unloading of heart, ↑ access (vasodilation) (Gertz and Kurgan, 1988), ↓ reperfusion injury by scavenging free radicals (McMurray et al., 1989) |
| Inhibitors of reperfusion damage (various) | Reviewed by Opie, 1989 |
| Lignocaine | ↓ Reperfusion arrhythmias (Marco et al., 1989) |
| Hyaluronidase | Angiogenesis |

with AMI after thrombolysis (Hogg et al., 1989), but the clinical demonstration of their superiority over aspirin in inhibiting platelet function may be a demanding test.

The requirement for heparin is still under discussion. For SK, the level of anticoagulation with heparin was inversely related to frequency of recurrent ischaemic events (Kaplan et al., 1987), but there are also SK-treated patients at risk for reocclusion who seem to be poorly protected by heparin (Eisenberg et al., 1986). Heparin plus SK produces less in-hospital mortality (SCATI Group, 1989), but the requirement for heparin, when aspirin is also used, remains to be established (ISIS-3 study). Heparin can influence both the basal enzymatic activity of t-PA and fibrin enhancement of plasminogen activation (Fears, 1989a), but initial clinical evidence (Topol et al., 1989) suggests that early patency is not affected.

Studies in which the interaction between SK and nitroglycerin (Rentrop et al., 1989) were examined showed an additive improvement in left ventricular function, but diverse mechanisms are possible (table 9). A beneficial effect of prostaglandin $E_1$ in combination with SK (Sharma et al., 1986) has been observed. Other cotherapies, such as β-adrenergic blockers and $Ca^{2+}$ antagonists, also are subjects for current experimental and clinical investigations. Initial results are confusing. For example, nifedipine did not improve outcome after SK treatment in patients (Erbel et al., 1986), whereas diltiazem enhanced salvage of reperfused ischaemic myocardium in dogs (Knabb et al., 1986). Angiotensin-converting enzyme inhibition has also been proposed as a potentially useful cotherapy and, again, diverse mechanisms are possible (table 9).

Whether or not reperfusion damage occurs after thrombolytic therapy is controversial (Anon, 1989). Clinical evidence is indirect and relies on such procedures as identifying the level of circulating neutrophils as a risk factor for ventricular fibrillation after AMI (Kawalsky et al., 1988) and variations in plasma superoxide dismutase activity as a risk factor for the extent of regional wall motion defects (Kodama etal 1989). The complication of reperfusion injury is discussed in detail elsewhere (Opie, 1989) and will not now be reviewed here, but current pharmacological approaches, such as the reduction of experimental infarct size by human soluble recombinant complement receptor type-1 (Weisman et al., 1989) in this area are of great interest. The implications if reperfusion injury exists are considerable, not just with regard to adjunct therapies but also with regard to choice of thrombolytic agent, because speed of reperfusion may be important in the induction of reperfusion injury (Opie, 1989).

Although hyaluronidase has not yet been examined as an adjunct therapy, clinical evidence suggests that hyaluronidase is beneficial in patients with AMI who demonstrate early spontaneous reperfusion (Roberts et al., 1988). Such benefit also may be related to an attenuation of reperfusion injury, but other effects have been observed experimentally. For example, stimulation of collateral blood flow and angiogenesis are of particular interest (Roberts et al., 1988). It has been suggested (Schwartz et al., 1984) that few patients with AMI initially show significant collateral development and that collaterals usually contribute little to the preservation of the myocardium during infarction. Therefore, pharmacological approaches to encourage new vessel development and function might be of great use in promoting recovery from ischaemia and in protecting against the consequences of reocclusion. Apart from hyaluronidase, neovascularisation can be induced by delivery systems bearing acidic or basic fibroblast growth factors (Thompson et al., 1988; Barath et al., 1988). Further developments in this area will require methods to maximise bioavailability and minimise side effects (confining angiogenesis to the myocardium).

## VI. Pharmacological Properties of Novel Plasminogen Activators

### *A. Requirements for the Next Generation of Thrombolytic Agents*

The design and testing of novel plasminogen activators is a very active research area, taking advantage of recent advances in genetic engineering techniques. The topic has been reviewed comprehensively by others (Krause, 1988; Krause and Tanswell, 1989; Lijnen and Collen, 1988). In this review examples have been selected (tables 10–12) to illustrate the types of enzymes constructed and the methods of assay used.

It has not always been obvious what will be expected of the new agents. Although the pharmacological attributes of high fibrin binding and good bioavailability have been seen as desirable, there is less agreement concerning the therapeutic weaknesses in the current generation of

agents that need to be overcome in the next generation. Doubts have been expressed that a long clearance half-life is necessarily an advantage (Bang, 1989; Krause and Tanswell, 1989): the benefit obtained may depend on the type of plasminogen activator. For example, increased bleeding has been observed when circulating levels of t-PA are maintained (Bang, 1989) but the long half-life of APSAC provides convenience of administration and is associated with a low reocclusion rate and high efficacy without excess adverse events. What is clear is that many groups are attempting to prolong half-life in novel activators (some are exemplified in table 10).

### *B. Improvement in Pharmacokinetics*

*1. Identification of t-PA domains involved in tissue uptake.* In a series of clearance studies in which the complications of inhibitor complex formation were avoided by temporary acylation of the active site (Dodd et al., 1988; Browne et al., 1989), it was observed that activator residence time in the circulation was prolonged by deletion of the growth factor and first Kringle domains on the A-chain. Other work suggests, alternatively, that the fibronectin-like finger domain may be involved in clearance (Lijnen and Collen, 1988; Larsen et al., 1989). Discrepancies among results from different groups of workers may arise because of differences in cell expression systems and in the precise construction of mutants (Pannekoek et al., 1988) as well as in the assay methods chosen to measure pharmacokinetics. Variations in rate of loss of circulating activity in different animal models may be determined by species differences in level of inhibitor, and methods based on radiometric or immunoassay procedures will measure inactive as well as active material in the circulation. For example, for the mutant protein in which finger and growth factor domains were deleted, the clearance of fibrinolytic activity by functional assay was more rapid than the clearance of total antigen (Kalyan et al., 1988). The identification of specific domains affecting pharmacokinetics is also difficult because deletion of a domain not directly involved may transmit structural changes to contiguous regulatory domains (Krause and Tanswell, 1989), that is, loss of specific function on erasure of a domain is not sufficient evidence for the normal function of that domain.

*2. Other activators.* Although there is no certainty about the precise regulatory sequences involved, domain-deletion studies may have established the upper limit for the slow plasma clearance of A-chain mutant proteins (Browne et al., 1989). If this maximum effect is perceived to be an insufficient pharmacological improvement, then alternative strategies for the synthesis of slowly cleared plasminogen activators must be contemplated, for example, hybrid enzymes composed of A-chain plasmin/B-chain plasminogen activators (table 10) (Robinson et al., 1988).

### *C. Thrombus Targeting*

A-chain plasmin/B-chain plasminogen activator hybrids also demonstrate high affinity for fibrin (table 11) (Robbins and Tanaka, 1986; Robbins and Boreisha, 1987) and catalytic efficiency is markedly enhanced by fibrin in model systems (Fears et al., 1990a). Representative kinetic results for hybrid enzymes comprising the A-chain of plasmin (Lys 78) and B-chain of t-PA (Ile 276) or u-PA (Ile 159) in response to the fibrin-mimetic CNBr-digested fibrinogen are shown in fig. 11. It can be concluded, therefore, that specific domains of the A-chain of t-PA are not necessary for ternary complex

TABLE 10
*Novel thrombolytic agents with slower clearance half-lives*

| Parent protease | Modification producing slower clearance | Comments | Reference |
|---|---|---|---|
| t-PA | A-chain domain deletions (active centre acylated) | Functional clearance in guinea pigs mediated by growth factor and $K_1$ domains | Browne et al., 1989 |
| t-PA | Growth factor domain deleted | Slow clearance in mice (antigen and functional assays) | Kalyan et al., 1988; Fu et al., 1989 |
| t-PA | Growth factor and fibronectin-like fingers deleted (remainder nonglycosylated) | Slow clearance (radiometric) in rats; slow clearance (antigen) and increased lytic activity in dogs | Larsen et al., 1989; Cambier et al., 1988 |
| tcu-PA/t-PA | Conjugation with polyethylene glycol | Slow functional clearance in guinea pigs | Garman and Kalindjian, 1987 |
| t-PA | Catalytic fragment bound to B fragment of staphylococcal protein A | Lytic activity in rabbit embolic stroke model (not compared to t-PA) | Phillips et al., 1989 |
| u-PA/t-PA | Replacement of A-chain by A-chain of Lys-plasmin (active centre acylated) | Slow clearance (30- to 100-fold change) in guinea pigs (functional assay) | Robinson et al., 1988 |
| scu-PA | Growth factor domain deleted | Slow clearance (radiometric) in rats | Hiramatsu et al., 1989 |

TABLE 11
*Novel thrombolytic agents with fibrin affinity*

| Parent protease | Sequence conferring fibrin affinity | Comments | Reference |
|---|---|---|---|
| scu-PA/tcu-PA | Fibrin-specific monoclonal antibody (64C5) | 10- to 1000-fold increase in potency in purified systems; less effect in plasma or rabbit thrombosis model | Bode et al., 1987 |
| t-PA | Fibrin-specific monoclonal antibody (59D8) | | Runge et al., 1988a |
| scu-PA/tcu-PA | Fibrin-specific monoclonal antibody (MA15C5) | 4- to 8-fold increase in potency in rabbit thrombosis model | Collen et al., 1989 |
| u-PA/t-PA | Replacement of A-chain by A-chain of Lys-plasmin | Bound and catalytic activity enhanced by fibrin in vitro | Robbins and Tanaka, 1986; Robbins and Boreisha, 1987; Fears et al., 1990a |
| u-PA | Addition of $NH_2$-terminal sequences of t-PA to COOH-terminal sequences of u-PA | Fibrin binding equal to or less than t-PA in vitro | Gheysen et al., 1987; Nelles et al., 1987, de Vries et al., 1988, Piérard et al., 1989; Lee et al., 1988 |
| tcu-PA | Lys-plasmin (conjugated at active centre) | Fibrin affinity similar to Lys-plasminogen; activity enhanced by fibrin | Ferres et al., 1988 |

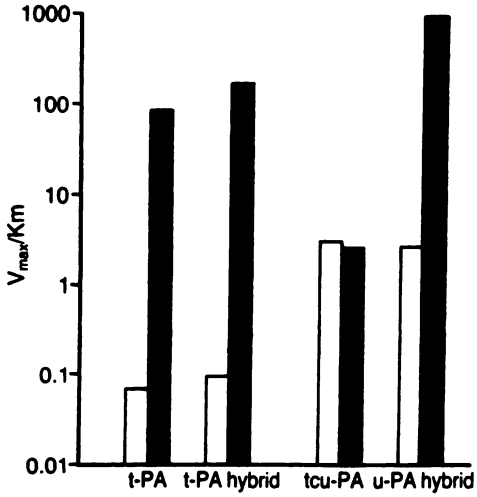


FIG. 11. Catalytic efficiency of novel hybrid plasminogen activators compared to parent activators. The efficiency of plasminogen activation (calculated as $V_{max}/K_m$) was measured in the absence and presence of CNBr-digested fibrinogen to simulate the influence of fibrin. For experimental details see Fears et al., 1990a.

formation among enzyme, substrate, and fibrin or for fibrin enhancement of catalytic activity. Ternary complexes can also be formed by enzymes bearing the A-chain domain of plasmin(ogen), for example, these hybrids, APSAC (section IV, D) and the novel activator comprising tcu-PA, conjugated with the active site of Lys-plasmin (table 11) (Ferres et al., 1988).

An alternative, novel approach to thrombus targeting derives from the work of Sakharov and colleagues (1988), whereby covalent conjugates of vector antibodies to fibrinogen with monoclonal noninhibitory antibodies to tcu-PA were used to confer affinity for tcu-PA to experimental clots. Preadministration of conjugate resulted in a 10-fold decrease in the level of tcu-PA required for clot lysis in experimental systems. Related work using a bispecific antibody to capture t-PA at the site of a fibrin deposit (Bode et al., 1989) also resulted in the promotion of the fibrinolytic potency of exogenous t-PA in vivo. The general approach may, therefore, both augment the contribution made by endogenous plasminogen activator to fibrinolysis and lead to a reduction in the amount of exogenous activator required but, if lower doses of activator could be used, then the possible amplification of the influence of endogenous antiproteases must be considered.

Improvements in fibrinolytic activity observed for a novel plasminogen activator often cannot be ascribed to specific changes in single pharmacological attributes. For example, the agent comprising a monoclonal antibody to cross-linked fibrin conjugated to u-PA (Collen et al., 1989) has both fibrin affinity (table 11) and a slower clearance than u-PA. In this instance, however, the longer residence time may not be of paramount importance because the homologue bearing an irrelevant antibody did not demonstrate greater potency despite its somewhat slower clearance (Collen et al., 1989).

### *D. Potential Benefits of Active Site Acylation*

Most of the current interest in novel activators has centred on the application of biotechnology in the design and expression of new enzymes. However, by analogy with APSAC (Smith et al., 1981), the temporary insertion of an acyl group into the catalytic centre of novel activators may confer extra benefits. Recent applications of the acyl-enzyme concept in the design of new proenzymes are exemplified by publications concerning t-PA mutant proteins (Browne et al., 1989) and plasmin/

activator hybrids (Robinson et al., 1988). Among the potential benefits of reversible, active centre acylation are control of tissue binding mediated by the B-chain sites, evasion of binding by plasma inhibitors, attenuation of systemic plasminogen activation, and the opportunity for rapid administration without immediate kinin generation and hypotension. Thus, in principle, active site acylation may increase potency, prolong circulating residence time, provide sustained action, and allow ease of administration.

### *E. Novel Plasminogen Activators as Pharmacological Tools*

It is too early to know whether any of the new agents in preclinical research will represent therapeutic advances but many are useful in advancing our understanding of the biochemistry of fibrinolysis. For example, the site-specific mutagenesis of scu-PA to devise analogues resistant to plasmin and thrombin (table 12) has been helpful in investigations of the mechanism of action of scu-PA, particularly with regard to the nature of fibrin selectivity and the requirement for conversion to tcu-PA (Lijnen and Collen, 1988). Similarly, mutagenesis of t-PA has been useful in examining the consequences of the one- to two-chain transition as well as to identify the domains involved in binding to fibrin (reviewed by Fears, 1989a) and inhibitors (table 13) (Madison et al., 1989; Monge et al., 1989). Whether or not addition of extra copies of (Kringle) domains that may mediate fibrin binding leads to a substantial increase in lytic activity (Stern et al., 1989; Urano et al., 1989) remains to be proven.

Site-specific mutagenesis of t-PA may also be of help in elucidating the influence of glycosylation status on function. This is an important topic because a set of glycoforms of the polypeptide is unique to the cell population in which it is expressed, and variations in the glycosylation of t-PA, according to cell source, may have therapeutic implications. For example, different glycoforms of t-PA differ in binding to plasminogen and fibrin (Parekh et al., 1989; Wittwer et al., 1989). Although it has been claimed (Spellman et al., 1989) that none of the glycoforms of t-PA produced in Chinese hamster ovary cells (source for the pharmaceutical material, Activase) would be expected to act as heterophile antigens immunogenic for man, there is the potential for creating novel epitopes or increasing levels of those that were previously subimmunogenic (Parekh et al., 1989). In particular, t-PA from Chinese hamster ovary cells, but not from human fibroblasts, possesses a significant number of oligosaccharides whose outer arms terminate in N-acetylglucosamine (Parekh et al., 1989). These are rare substituents in circulating glycoproteins and the potential consequences of their introduction include complex formation with naturally occurring anti-N-acetylglucosamine antibodies, activation of monocytes, and interaction with specific cellular receptors.

### *F. Anticipated Developments*

It has been concluded (Bang 1989; Runge et al., 1988) that, as yet, there is little published evidence to show that any of the novel plasminogen activators possess increased potency compared with the standard thrombolytic agents. Thus, even when fibrin affinity or a prolonged circulating residence time is gained, there may be decreases in specific activity or protein stability. However, because of the many differences between experimental models and the clinical state (for example, regarding accessibility of thrombus, level of inhibitors, stimulus to reocclusion, risk of bleeding), it will always be difficult to forecast therapeutic activity. It may be necessary, therefore, to proceed on the basis of confidence in the acquisition of the desired pharmacological attributes, for example, high thrombus affinity and sustained action (section V). New agents with these features are expected to enter clinical development soon (Bang et al., 1989).

Progression of novel thrombolytic agents will be accompanied by developments in adjunct therapies (see section V, B, 6). In particular, the ability of plasminogen activators to achieve thrombus dissolution may be promoted by interference with platelet-mediated events, by new pharmacological approaches (platelet receptor antibodies and antagonists, thromboxane synthetase inhib-

TABLE 12

*Other novel thrombolytic agents with altered pharmacological properties*

| Parent protease | Modification | Comments | Reference |
|---|---|---|---|
| scu-PA | Point mutations at plasmin cleavage site | Attempt to enhance fibrin selectivity but poor lytic activity observed in vitro | Reviewed by Lijnen and Collen, 1988 |
| t-PA | $K_1K_2$ domains deleted | Decreased inhibition by PAI-1; modest increase in lytic activity in vitro | Ehrlich et al., 1987 |
| t-PA | Active site Ser 195 mutants | Ser → Thr substitution retained slight enzymic activity in vitro | Monge et al., 1989 |
| t-PA | Site-directed mutagenesis (residues 296-304) | Resistant to inhibition by PAI-1 in vitro | Madison et al., 1989 |

itors, and receptor antagonists), and with anticoagulation by specific thrombin inhibitors and novel anticoagulants such as protein C (reviewed by Bang et al., 1989).

## VII. Conclusions and Future Directions

There is consensus that thrombolytic therapy for AMI can salvage the myocardium and improve survival. There is controversy as to whether all such benefits derive from the early restoration of patency or whether late opening and ancillary pharmacological actions (such as, reducing viscosity) also may contribute. Some of the other key questions that still need to be answered concern who should be treated (extending therapy to those with relatively late onset of symptoms) and where treatment can be given (extending therapy prior to admission to hospital).

There is reason to expect that thrombolytic agents also may offer benefit in other thrombotic disorders, where timely restoration of oxygen and nutrients will salvage ischaemic tissue. Although it has not been possible to review these other thrombotic states, there is considerable experience in the use of thrombolytic agents in pulmonary embolism, deep vein thrombosis, and peripheral arterial disease. Trials are also defining efficacy in stroke and unstable angina and further extensions of therapy are likely.

The properties of the individual plasminogen activators have been examined in this review. Desirable pharmacological attributes (pharmacokinetic properties and fibrin affinity) can be delineated by reference to clinical objectives in treating AMI (high potency, sustained action, convenience of administration). There is, as yet, no consensus concerning which standard thrombolytic agent may be preferred (Chamberlain, 1989). t-PA is the most fibrin selective in the sense of demonstrating the least effect on circulating fibrinogen levels, and scu-PA probably also possesses some fibrin selectivity in current dosage regimens. However, fibrin selectivity does not equate to thrombus selectivity and all the agents are probably indistinguishable as regards their effect on bleeding. APSAC has the longest half-life, and this pharmacological feature is associated, clinically, with sustained action and convenience of administration. The effect of APSAC in reducing mortality also appears greater than the other standard agents, but definitive proof must await the conclusions from the ongoing direct comparisons and a further understanding of the effects of adjunct agents. The choice of appropriate adjunct therapy is attracting considerable attention not only with respect to confirming the benefits of established anticoagulant and antiplatelet agents but also regarding novel complementary strategies, for example, attempting to attenuate reperfusion injury.

Following the general acceptance of the impressive therapeutic value of thrombolytic agents and the advances made in defining desirable pharmacological features, there is enthusiasm to design novel plasminogen activators. Initial progress has been made in establishing structure-activity relationships but much remains to be done and it will not be easy to direct activator to the site of atherosclerotic injury or to promote endogenous fibrinolysis.

The early mortality rate after AMI was as high as 30% before the introduction of coronary care units, declining to a current rate of about 12% in the absence of thrombolytic therapy. A rate as low as about 6% in appropriate populations can now be achieved by using a thrombolytic agent. Further improvement on this remarkable progress by developing novel plasminogen activators and adjunct therapies represents a considerable challenge in pharmacological research.

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