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Review

Recombinant Tissue Plasminogen Activator: A Brief Review

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Thrombolytic therapy has received renewed attention with the demonstration that early treatment of acute myocardial infarction with plasminogen activators can reduce mortality. Tissue plasminogen activator (t-PA) is a protein with attributes that may allow for greater efficacy and safety. Recombinant DNA technology has enabled the production of sufficient t-PA, called rt-PA (Activase), for substantial clinical evaluation. The results suggest that Activase is a significant advance in thrombolytic therapy.

KEY WORDS: thrombolytic; tissue plasminogen activator; plasminogen activators; coronary thrombolysis.

INTRODUCTION

Recent clinical studies demonstrating that a coronary thrombosis is the precipitating event in acute myocardial infarction and that, not surprisingly, coronary thrombolysis has a beneficial effect on the clinical outcome of acute myocardial infarction have stimulated great interest in the properties and clinical efficacy of a new thrombolytic drug, recombinant tissue plasminogen activator (rt-PA; Activase). Since no generic name is available for rt-PA yet, and since virtually all published data on clinical trials refer to rt-PA, this review focuses on relevant studies on the biology, clinical pharmacology, and efficacy of Activase.

BIOLOGY OF PLASMINOGEN ACTIVATION (1) (FIG. 1)

The central step in the fibrinolytic system is conversion of the proenzyme plasminogen to the active enzyme plasmin, a relatively nonspecific serine protease with trypsin-like specificity. Plasminogen is normally included in thrombi as they are formed; thus if activated *in situ,* the plasmin dissolves the fibrin without systemic fibrinogen breakdown.

Activated plasmin is generated by two proteolytic events, the cleavage of an Arg–Val bond near the carboxyl terminus of the molecule resulting in a two-chain molecule held together by a single disulfide bone and the removal of an amino-terminal peptide to yield the more active lys-plasmin. Once the active moiety, plasmin, has been generated, it becomes a relatively nonspecific enzyme with a high catalytic activity. Systemic plasminogen activation leads to the degradation of fibrinogen, Factor V, and Factor VIII and is accompanied by the appearance of fibrinogen breakdown products with their own anticoagulant properties.

The main inhibitor of plasmin in blood is α-2 antiplasmin, which is known to compete for the fibrin binding sites of plasmin. α-2 Antiplasmin is a single-chain glycoprotein of molecular weight 70,000 which forms a one-to-one stoichiometric complex with plasmin while undergoing proteolysis and is the body's primary defense against systemic degradation of the hemostatic system. During thrombolytic therapy the free inhibitor may disappear due to its reaction with plasmin. A less specific inhibitor with less affinity, α-2 macroglobulin, provides the hemostatic system with a second line of defense. Plasmin generated at the site of a clot (and therefore bound to fibrin) reacts far more slowly with inhibitors than plasmin in circulation since the fibrin binding sites of bound plasmin are relatively unavailable (2).

Tissue Plasminogen Activator (t-PA)

Native t-PA as isolated from a melanoma cell line is a single-chain glycoprotein protease of molecular weight 65,000. The single-chain molecule is susceptible to enzymatic digestion to a two-chain molecule in which the two chains remain covalently linked by a disulfide bond. Cloning of the cDNA from melanoma cells has led to the deduction of an amino acid sequence (527 residues) (3). The heavy or A chain (of the two-chain form) contains the fibrin binding region. The light (or B) chain contains the catalytic site, with homology to other serine proteases.

Glycosylation microheterogeneity (Type I and Type II t-PA) leads to an apparent molecular weight difference of 3000 in the A-chain or the single-chain molecule. This difference has been ascribed to the presence or absence of carbohydrate moieties at position 184 on the A chain of t-PA. The activator has three other potential N-linked glycosylation sites, two of which are known to contain carbohydrate residues.

The one-chain and two-chain forms have virtually the same fibrinolytic and plasminogen activating properties, and it has been suggested that conversion of the one-chain to the two-chain form occurs rapidly (as a result of traces of plasmin) at the fibrin surface, such that physiologic fibrinolysis is mainly a result of two-chain moiety (4). Tissue plasminogen activator appears to have considerable clot specificity as evidenced by a striking fibrin-specific enhancement

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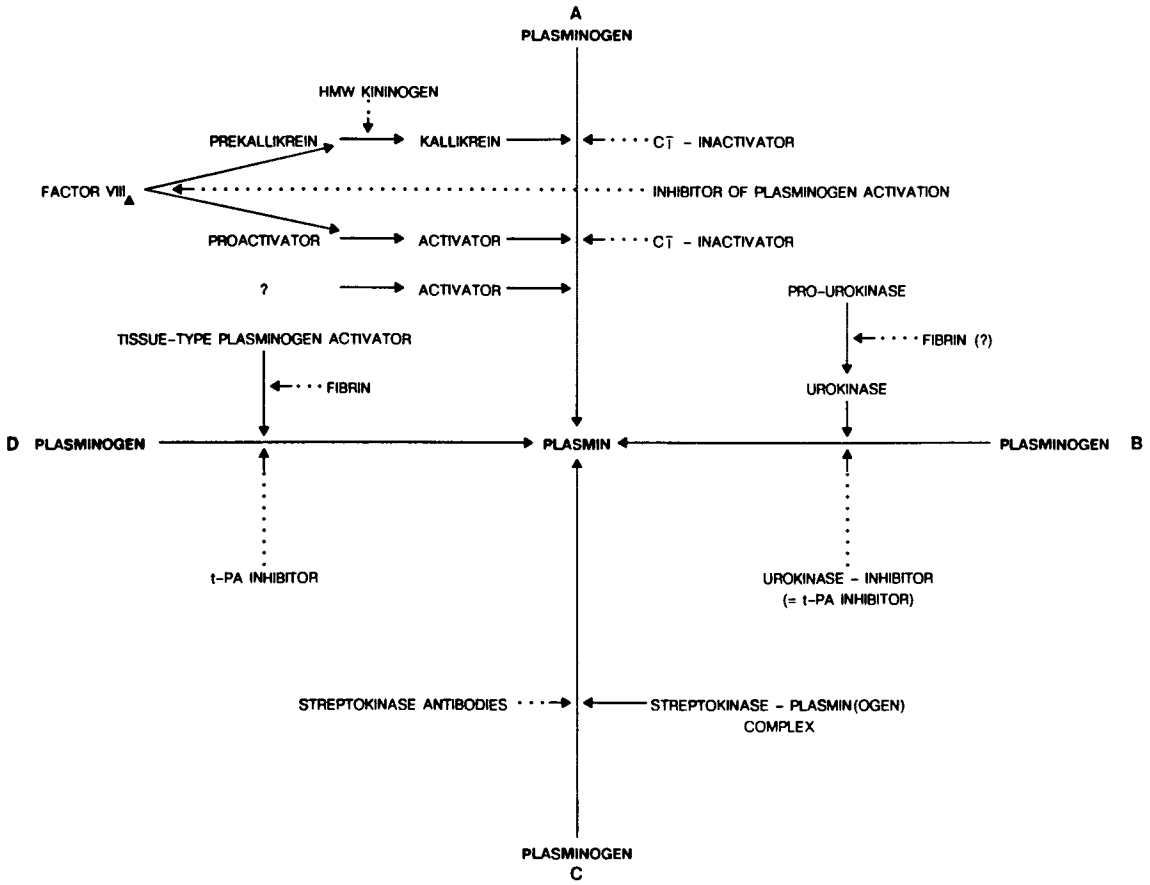

Fig. 1. Plasminogen activation occurs via a number of pathways, each of which may have specific inhibitors associated with it. Endogenous activators include t-PA, pro-urokinase, urokinase, and the kallikrein system. Streptokinase is an (indirect) exogenous activator.

of plasminogen activation by t-PA. Thus t-PA is a poor enzyme in the absence of fibrin, but in the presence of fibrin (the primary constituent of a thrombus), its plasminogen activating capacity is substantially increased, allowing efficient activation at the clot but not in the circulating plasma.

Pharmacokinetics

Initial clinical trials with Activase utilized a predominantly two-chain material (G11021), while subsequent studies have been with a predominantly one-chain material (G11044). Pharmacokinetic studies suggest that the latter material undergoes more rapid clearance from the circulation (5). Both preparations show a biexponential clearance, with a short $t_{1/2}$ α (5 min) and a longer $t_{1/2}$ β. The clearance of Activase is of the order of 500–600 ml/min and the VD_{ss} is approximately 10 liters (6) (Tables I and II). The pharmacokinetic differences observed between G11021 and its successor product are *not* due to the different one-chain to two-chain ratios of the two products (6). The different clearance rates were associated with different efficacies and thus, however, required a modification in the dose required to effect coronary thrombolysis (vide infra).

CLINICAL STUDIES WITH ACTIVASE

Rationale

The rationale for thrombolytic therapy is based upon the thesis that rapid reperfusion of ischemic myocardium restores oxygen and nutrients to sufficient myocardium to result in improved ventricular function (over that which would exist in the absence of coronary thrombolysis) (7). Ventricular function correlates with survival following myocardial infarction. Thus coronary thrombolysis should reduce mortality following myocardial infarction, a hypothesis that has been recently validated (8,9).

Table I. Pharmacokinetic Parameter Estimates Following a 0.25-mg/kg rt-PA Infusion over 10 min in Eight Patients with Thrombooclusive Disease[a]

	Mean	±SD	Range
Two-compartment model			
$t_{1/2}$ α (min)	4.36	0.94	3.37–5.77
$t_{1/2}$ β (min)	26.5	11.0	15.0–47.5
V (liters)	3.82	1.40	1.69–6.11
AUC (ng—min/liter)	36.3	11.9	21.0–55.8
CL (ml/min)	549	180	269–926
Compartment—independent			
AUC (ng—min/liter)	34.3	8.98	23.2–49.3
VD_{ss} (liters)	10.3	3.05	5.36–15.1
CL (ml/min)	554	175	340–861

[a] Reproduced with permission from Ref. 6.

Table II. Comparative Pharmacokinetics of Three Preparations of rt-PA in Normal, Adult Male Human Subjects[a]

	Group 1 (N = 9)	Group 2 (N = 9)	Group 3 (N = 9)
$t_{1/2}$ α (min)			
Mean	4.16	3.93	3.60
SD	1.01	0.69	1.38
$t_{1/2}$ β (min)			
Mean	30.1	36.0	28.0
SD	12.0	15.7	14.9
V_1 (liters)			
Mean	4.31	4.33	4.25
SD	0.92	1.11	1.48
AUC (ng—min/liter)			
Mean	28.8	26.7	27.1
SD	5.10	4.81	5.35
VD_{ss} (liters)			
Mean	9.28	12.04	10.95
SD	2.40	7.13	4.21
CL (ml/min)			
Mean	630	730	690
SD	140	150	140

[a] Reproduced with permission from Ref. 6. No statistically significant differences by one-way ANOVA.

Efficacy (Table III)

Dose Selection

Initial clinical studies with Activase focused on selecting a dose that could reliably produce coronary thrombolysis in infarct-related arteries that had been demonstrated to be occluded prior to treatment (rather than to infer success based on the patency of infarct-related arteries studied only following therapy). These trials (utilizing G11021) showed that a dose of 50 mg (0.625–0.75 mg/kg) over 90 min was effective in approximately 70% of patients (10–12). During these studies it appeared that due to a variety of factors including severe residual stenosis and residual thrombus, the incidence of rethrombosis or reocclusion might be as high as 20 to 30%. Studies since that time have suggested that by lengthening the infusion (total duration, 5–6 hr) and increasing the dose (increment of 40–80% over lysis dose), the rate of reocclusion can be reduced substantially (13,14), although one randomized trial shows a reocclusion rate below 10% with or without a maintenance infusion (15).

Table III. Major Clinical Trials with Activase

Ref. No.	No. studied	Dose[a]/duration[b]	Product code	Efficacy (%), p or r[c]
10	50	40–60/1–2	G11021	75 (r)
11	143	80/3	G11021	66 (r)
12	64	60/1.5	G11021	70 (p)
16	73	100/3	G11044	70 (p)
17	153	100–150/3–6	G11044	70–75 (r)
19	386	150/6	G11044	75 (p)

[a] Milligrams (in patients treated according to weight; assume 80 kg).
[b] Hours.
[c] p, patency at 90 min (no preintervention angiography); r, reperfusion at 90 min (preintervention angiography showing occlusion).

Comparison with Streptokinase

Streptokinase had been used in the major trials that had demonstrated clinical benefit from coronary thrombolysis (8,9), and therefore, two randomized trials were conducted to compare intravenous Activase to intravenous streptokinase. Both studies showed a decided (15–30%) advantage for Activase (11,12). The TIMI trial difference was so great ($P < 0.001$) that it was terminated earlier than planned. An additional trial comparing iv Activase to intracoronary streptokinase showed that the intravenous administration of Activase was a tremendous advantage in achieving reperfusion promptly (16).

Commercial-Scale Activase and Dose Response

The scale-up of Activase production required manufacturing adjustments that in turn generated a final product (G11044) with different pharmacokinetic properties (a 30–40% more rapid clearance). Dose–response trials conducted with this product demonstrated that, consistent with the more rapid clearance, the lysis dose, that dose which is administered over the first 60 to 90 min to effect coronary thrombolysis, needed to be increased about 50% (to 70 mg). Furthermore, if the lysis dose were increased further (to 100 mg), the time to reperfusion could be shortened, although the overall efficacy was unchanged (17). The benefit of more rapid reperfusion might be offset if the high dose was associated with an increased risk (vide infra).

Ongoing Studies with Activase

Recent studies take cognizance of the fact that thrombolytic therapy is becoming standard therapy for appropriate patients who are in the first few hours of an acute myocardial infarction. The major trials under way now seek to assess the utility and timing of coronary angioplasty following Activase therapy, the value of β-blocker therapy following Activase, and the potential value of synergistic combinations of thrombolytic agents (18–20).

Safety

The most significant risk associated with treatment with Activase is bleeding. Activase treatment does not appear to be associated with allergic or hypotensive reactions. The incidence of formation of antibody to Activase following treatment is less than 0.4% (3 of over 700) and the antibody disappeared subsequently in all three patients.

The evaluation of bleeding has been complicated by the subjective nature of the observation, by the coadministration of heparin, and by the almost uniform use of invasive procedures (arterial puncture, catheterization, coronary bypass grafting). Nevertheless, it is clear that oozing from puncture sites or recent wounds is to be expected during Activase therapy.

The risk of bleeding is related to two features of thrombolytic therapy. The first is the nonspecific fibrinogenolytic effects of systemic hyperplasminemia. Since Activase is clearly more fibrinogen-sparing than streptokinase (12), it

would be expected that bleeding caused or exacerbated by fibrinogen depression would be reduced by treatment with Activase, as opposed to streptokinase. The second factor that could produce bleeding is the heightened lytic activity in the blood when pharmacologic concentrations (up to 1000-fold greater than normal) of a physiologic substance are present. The short half-life of Activase helps to mitigate this factor. Discontinuation of therapy leads to a rapid reduction in Activase concentrations.

Life-threatening spontaneous bleeding, particularly intracranial, occurs infrequently, but there is evidence that it may be dose related. Risk factors for intracranial bleeding include advanced age, severe peripheral vascular disease, a history of poorly controlled hypertension or severe hypertension (diastolic pressure, >110 mm Hg), and previous cerebrovascular accident or even transient ischemic attack. At doses up to 100 mg the incidence of intracranial bleeding is approximately 0.3% (2000 patients). At a dose of 150 mg the incidence of such bleeding may be as high as 1–1.5% (1000 patients) (21,22). It should be noted that one comprehensive review of randomized myocardial infarction thrombolytic therapy trials reports a 0.6% incidence of cerebrovascular accident in nonthrombolytic treated controls (23).

Studies in Other Indications

Pilot studies have been initiated for a variety of other indications in which the pathophysiology of the disease involves thromboocclusive disease of the vasculature. Substantial efficacy (thrombolysis) has been reported in pulmonary embolism (24), peripheral occlusion (25), venous thrombosis (26), and unstable angina (27). The risk-benefit ratio for each of these conditions will need to be considered independently and appropriate trials will need to be conducted.

Reconstitution and Dilution of Activase

In the clinical setting (i.e., iv bag–iv set) Activase can be reconstituted with sterile water for injection (no preservative) to 1.0 mg/ml and then maximally diluted to 0.2 mg/ml with 0.9% sodium chloride injection. The solutions are physically and chemically stable for 24 hr at ambient temperature. Activase can also be reconstituted with 0.9% sodium chloride injection and then diluted to 0.5 mg/ml with the same. Reconstitution of Activase with water for injection and further dilution to less than 0.2 mg/mL with 0.9% sodium chloride injection are not recommended.

CONCLUSION

Advances in recombinant technology and tissue culture have afforded cardiologists a superior thrombolytic drug for use in the aggressive treatment of myocardial infarction. Despite impressive progress to date and a clinical experience in excess of 4000 patients, much remains to be learned about the optimal use of Activase. Major clinical trials in several indications are under way.

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