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TISSUE-TYPE PLASMINOGEN ACTIVATOR

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INTRODUCTION

Thrombotic complications of cardiovascular diseases, such as acute myocardial infarction, cerebrovascular thrombosis and venous thromboembolism, constitute a main cause of death and disability. Thrombolytic therapy, which consists in the administration of plasminogen activators which activate the fibrinolytic system in blood, could favorably influence the outcome of these life-threatening diseases. The fibrinolytic system contains a proenzyme, plasminogen, which by the action of plasminogen activators is converted to the active enzyme plasmin, which in turn digests fibrin to soluble degradation products. Inhibition of the fibrinolytic system occurs both at the level of the plasminogen activators, by plasminogen activator inhibitors (mainly PAI-1 and PAI-2) and at the level of plasmin, mainly by α_2-antiplasmin.

Currently, five thrombolytic agents are either approved for clinical use or under clinical investigation in patients with acute myocardial infarction. These include streptokinase, two chain urokinase (tcu-PA), anisoylated plasminogen streptokinase activator complex (APSAC), tissue-type plasminogen activator (t-PA) and single chain urokinase-type plasminogen activator (scu-PA, prourokinase). Streptokinase, APSAC and two chain urokinase cause extensive systemic activation of the fibrinolytic system, which may result in degradation of several plasma proteins, including fibrinogen, Factor V and Factor VIII.

Physiological fibrinolysis, however, is highly fibrin-specific as a result of molecular interactions between the components of the fibrinolytic system (1). The physiological plasminogen activators, t-PA and scu-PA, activate plasminogen preferentially at the fibrin surface. Plasmin, associated with the fibrin surface, is protected from rapid inhibition by α_2-antiplasmin and may thus efficiently degrade the fibrin of a thrombus (1,2). This fibrin-specific mechanism of action of t-PA has triggered great interest in the use of this agent for therapeutic thrombolysis. Production of t-PA by recombinant DNA technology (rt-PA) (3,4) has made it available for large scale clinical use. This chapter will review the biochemical and biological properties of rt-PA and its use for thrombolysis.

BIOCHEMICAL PROPERTIES OF TISSUE-TYPE PLASMINOGEN ACTIVATOR (t-PA)

Sources of t-PA

The first satisfactory purification of human t-PA was obtained from uterine tissue (5). Using an antiserum raised against uterine plasminogen activator, it was shown that tissue plasminogen activator, vascular plasminogen activator, and blood plasminogen activator are immunologically identical, but different from urokinase (6). The plasminogen activator found in blood is identical to vascular plasminogen activator, which is synthesized and secreted by endothelial cells (7) and is now generally called "tissue-type plasminogen activator" (t-PA). t-PA has been purified from the culture fluid of a stable human melanoma cell line (Bowes, RPMI-7272) in sufficient amounts to permit the study of its biochemical and biological properties (8).

The cDNA of human t-PA has been cloned and expressed in E. coli and in mammalian cell systems (3,9,10). The generation of Chinese hamster ovary cells capable of producing human t-PA has allowed the development of large-scale tissue culture fermentation and purification procedures (11). The resulting product (Activase^{R}) consists primarily of a single chain molecule. t-PA for clinical use is presently produced by recombinant DNA technology (Activase^{R}, Genentech Inc. or Actilyse^{R}, Boehringer Ingelheim GmbH).

Structural properties of t-PA

t-PA is a serine proteinase with a molecular weight of about 70,000, consisting of a single polypeptide chain of 527 amino acids with Ser as the NH_2-terminal amino acid, as deduced from the cDNA sequence (3). Plasmin converts t-PA to a two chain molecule by hydrolysis of the Arg^{275}-Ile^{276} peptide bond. The NH_2-terminal region (heavy chain) is composed of multiple structural-functional domains, including a "finger-like" domain (F) homologous to the finger domains in fibronectin, an "epidermal growth factor domain" (E) homologous to human epidermal growth factor, and two disulphide bonded triple loop structures commonly called "kringles" (K_1 and K_2), homologous to the kringle regions in plasminogen (3). The COOH-terminal region (light chain), comprising residues 276 to 527, is homologous to other serine proteinases and contains the catalytic site, which is composed of His^{322}, Asp^{371} and Ser^{478} (3). The structures required for the enzymatic activity of t-PA are fully comprised within the COOH-terminal polypeptide chain, as evidenced by the intact activity of the isolated chain, separated chemically (12,13) or prepared by recombinant DNA technology (14,15).

These structural domains of t-PA are involved in most of its functions and interactions, including its enzymatic activity, binding to fibrin, stimulation of plasminogen activation by fibrin, binding to receptors, and inhibition by plasminogen activator inhibitors.

Functional properties of t-PA

The structures involved in the fibrin-binding of t-PA are fully comprised within the NH_2-terminal (heavy) chain, as evidenced by the intact fibrin-affinity of the heavy chain isolated after mild reduction of two chain t-PA (12,13). Evidence obtained with domain deletion mutants of t-PA indicated that its affinity for fibrin is mediated via the finger domain and mainly via the second kringle domain (16). A lysine-binding site is involved in the interaction of the kringle-2 domain but not of the finger domain with fibrin (14). Gething, et al. (17) have however suggested that the kringle-1 and kringle-2 domains of t-PA would be equivalent in their affinity for fibrin, although the kringle-1 domain does not contain a lysine-binding site. The presence of a weaker lysine-binding site in kringle 2 similar to the "AH-site" (aminohexyl-site) in plasminogen has also been suggested (18). This AH-site would interact with internal lysine residues in the fibrin matrix, whereas the lysine-binding site would interact with COOH-terminal lysine residues. In the process of fibrinolysis, binding of t-PA to intact fibrin may initially be mediated by the F domain, while upon partial fibrin digestion by plasmin, increased binding of t-PA to newly exposed COOH-terminal lysine residues may occur via the lysine-binding site in the K_2 domain (16). Because of its AH site, the K_2 domain may also play a role in the initial binding to intact fibrin.

Mechanism of action of t-PA

t-PA is a poor enzyme in the absence of fibrin, but the presence of fibrin strikingly enhances the activation rate of plasminogen (19). The kinetic data support a mechanism in which fibrin provides a surface to which t-PA and plasminogen adsorb in a sequential and ordered way yielding a cyclic ternary complex. Fibrin essentially increases the local plasminogen concentration by creating an additional interaction between t-PA and its substrate. The high affinity of t-PA for plasminogen (low K_m) in the presence of fibrin thus allows efficient activation on the fibrin clot, while plasminogen activation by t-PA in plasma is a comparatively inefficient process (19). However, others have claimed that fibrin influences both the K_m and k_{cat} of the activation of plasminogen by t-PA (20).

Plasmin formed on the fibrin surface has both its lysine-binding sites and active site occupied and is thus only slowly inactivated by α_2-antiplasmin (half-life of about 10-100 s); in contrast, free plasmin, when formed, is rapidly inhibited by α_2-antiplasmin (half-life of about 0.1 s) (2). The fibrinolytic process thus seems to be triggered by and confined to fibrin.

THROMBOLYTIC PROPERTIES OF t-PA

In 1981, the first patients were treated with t-PA obtained from the Bowes melanoma cell line. Intravenous administration of 7.5 mg t-PA over 24 hours

induced complete lysis of a renal and iliofemoral thrombosis in a renal transplant patient without systemic fibrinolytic activation or bleeding (21). However, intravenous infusion of 5 to 25 mg of t-PA over 24 to 36 hours did not produce thrombolysis in four patients with deep vein thrombosis (22). A pilot-study of melanoma cell t-PA was carried out in 7 patients with acute myocardial infarction. Coronary thrombolysis in the absence of fibrinogen breakdown was achieved in 6 of these 7 patients (23).

These encouraging initial results have triggered the organization of clinical trials using recombinant t-PA in several indications, including deep vein thrombosis, major pulmonary embolism, arterial thromboembolism and acute thromboembolic stroke. Most experience has, however, been obtained in the treatment of coronary artery disease. Reduction of infarct size, preservation of ventricular function and/or decreased mortality have been demonstrated in patients with acute myocardial infarction following administration of streptokinase, APSAC and rt-PA (for references, cfr. 24,25). This beneficial effect of thrombolysis will probably also hold for other plasminogen activators.

Coronary reperfusion is very likely the most important, if not the only, significant contributor to preservation of ventricular function and to reduction in mortality, although the magnitude of the effect appears to be time dependent. Indeed, subgroup analyses of patients with and without successful reperfusion with streptokinase or rt-PA have consistently demonstrated a lower mortality rate in successfully reperfused patients, and a better outcome if reperfusion is obtained early. The most rational treatment of patients with acute myocardial infarction is therefore likely to be thrombolytic therapy with agents that reperfuse the most coronary arteries as rapidly as possible.

The 2 comparative trials to date which have examined the reperfusion efficacies of rt-PA and streptokinase have been evaluated by meta-analysis (26). These results show that rt-PA appears more effective than streptokinase for the early recanalisation of occluded coronary arteries, both when given within 3 hours of the onset of symptoms and when given later. Comparable effects on the preservation of left ventricular function have been demonstrated for rt-PA and streptokinase in 2 comparative trials, but futher studies would assist in determining whether differences in early coronary reperfusion rates may translate into a comparably better outcome. Much larger, direct comparative studies are needed before scientifically valid statements can be made regarding the relative efficacy of the available thrombolytic agents for the reduction of both moribidity and mortality. Such studies, i.e. GISSI-II are presently carried out.

ADMINISTRATION SCHEMES OF rt-PA

Due to its short in vivo half-life (initial $t_{\frac{1}{2}}$ of 4 min and terminal $t_{\frac{1}{2}}$ of 46

min in patients with acute myocardial infarction (27)) relatively large amounts of rt-PA have been administered as a continuous intravenous infusion. In clinical trials in patients with acute myocardial infarction, total doses of rt-PA ranging between 40 and 150 mg, have been administered. The currently used or recommended dose is 100 mg, to be given intravenously as a 10 mg bolus in 1 to 2 minutes followed by 50 mg in the first hour, 20 mg over the second hour and 20 mg over the third hour. For patients weighing less than 65 kg, a dose of 1.25 mg per kg, administered over 3 hours as indicated above, may be used. A dose of 150 mg has been associated with an increased frequency of intracranial bleeding and should not be used.

Neuhaus et al. (28) have administered an accelerated dosage regimen of rt-PA (15 mg intravenous bolus, 50 mg infusion over 30 min and 35 mg infusion over the following 60 min) to patients with acute myocardial infarction of less than 6 hours duration. Patent infarct-related arteries were observed in 74% of the patients at 60 min after the start of the infusion, in 91% at 90 min and in 92.4% at 24 hours. Rapid infusion of 100 mg rt-PA over 90 min thus yielded a high early patency rate, and was not associated with an increase in reocclusion or adverse effects (28).

Bolus administration of rt-PA has also been performed in patients with acute myocardial infarction. Tebbe et al. (29) have given a single 50 mg bolus of rt-PA over 2 min to 20 patients and obtained patency in 75% of those patients. Reocclusion occurred in 22% of patients and 1 patient died due to intracranial hemorrhage. rt-PA plasma levels increased to 9.8 $\pm$ 3.6 μg/ml and the bolus caused a decrease of circulating fibrinogen to 55% of baseline after 2 to 4 hours. Tranchesi et al. (30) have administered rt-PA as a bolus of 50 mg, 60 mg or 70 mg and concluded that a bolus of 70 mg yielded results comparable to the conventional infusion scheme (72% recanalization at 60 min), and was associated with only minor bleeding complications. These preliminary results in patients with acute myocardial infarction suggest that bolus administration of t-PA may yield similar patency rates as the infusion regimen.

Combined administration of t-PA and scu-PA has been performed in patients with acute myocardial infarction in order to investigate if the effect on clot dissolution might be more than additive. Synergism may allow a reduction of the total dose of thrombolytic agent and may reduce the occurrence of haemostatic side effects. Preliminary results in a small number of patients (31,32) have suggested that combining t-PA and scu-PA or tcu-PA at approximately 20% of their individual therapeutic dose, produced coronary artery reperfusion without associated fibrinogenolysis. Such synergistic effect of t-PA and tcu-PA on coronary reperfusion has, however, not been confirmed in a larger study, although the use of the combination was associated with a reduction of the frequency of reocclu-

sion (33). A subsequent study in patients with acute myocardial infarction, using 20 mg rt-PA combined with 10, 15 or 20 mg rscu-PA given intravenously over 90 min, yielded recanalization at 90 min in only 31 to 41% of patients (34). Combined administration at reduced doses thus was less efficient than that of either drug at the presently recommended dose.

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Discussion - TISSUE-TYPE PLASMINOGEN ACTIVATOR

P. Juul

Can you comment on the relationship between time of administration of t-PA and clinical response?

H.R. Lijnen

Already some of the earlier clinical trials suggested that it is very important to give the thrombolytic agent as soon as possible after the onset of myocardial infarction. It was then suggested that six hours after the onset of acute myocardial infarction t-PA would not be useful anymore to recanalize an occluded coronary artery. Several studies now underway are evaluating the effect of late recanalization of coronary arteries on clinical outcome.

M.M. Reidenberg

What is the difference between the thrombus that readily lyses and the ones that apparently do not even where the t-PA is given shortly after the clinical event?

H.R. Lijnen

Well, one of the possibilities is that a thrombus that is very rich in platelets would be much more resistant to thrombolysis. It is also known that an older thrombus is much more resistant to lysis than a younger thrombus, probably because of a higher extent of organization.

M.M. Reidenberg

But does organization occur within the first few hours?

H.R. Lijnen

Probably not.

M.M. Reidenberg

But yet this is the time period we are talking about. One cannot claim that a 30% failure in recanalization is due to the organization of the thrombus.

H.R. Lijnen

No. That's probably because thrombi which are very rich in platelets are very

resistant to lysis. Actually it may be that recanalization in 100% of patients is an impossible goal. There may always be 20% or 25% of patients that we will not be able to recanalize because of the resistance of the thrombus.

J. Bigorra

Do you have separate data for patients older than 60 or 65? What was the upper limit in this trial?

H.R. Lijnen

In the first clinical trials with t-PA the age limit was usually 65 to 75 years. In the GISSI II trial, however, more than 20 percent of the patients were over 70. The mortality in patients over 70 was much higher, but was not different between t-PA and streptokinase.

H.J. Röthig

Do you think the decrease in the levels in fibrinogen is bad in general? This reduces viscosity and most of the cardiologists are very keen on finding drugs which are doing this and probably this might be one of the advantages of streptokinase therapy?

H.R. Lijnen

A lot of people feel that to spare fibrinogen in a thrombolytic therapy is not really an advantage. And that there is no real correlation between frequency of bleeding complications and reduction of fibrinogen.

P. Tanswell

I think that the supposition that the use of streptokinase reduces blood viscosity is not based on fact. Although streptokinase does completely degrade fibrinogen this does not have a significant effect on blood viscosity. And the other point to be made regarding fibrinogen specificity is that this probably is responsible for the higher efficacy of t-PA even if a significantly lower bleeding tendency has not yet been fully demonstrated.

H.R. Lijnen

There is a difference in the nature of the fibrinogen degradation products that one gets with t-PA and streptokinase. With t-PA one gets much more early degradation products, the large X and Y fragments which can still clot but which are more slowly

coagulable. With streptokinase therapy one gets much smaller further degradation products of fibrinogen.

H.J. Röthig

Couldn't the high unphysiological doses of t-PA used in therapy induce the production of antibodies that would neutralize the effect of t-PA if it had to be used later, in the case of a second infarction?

H.R. Lijnen

The doses of t-PA used in therapy are very high, about 500-fold above the physiological level, but to my knowledge t-PA administration has never been associated with generation of antibodies. On the other hand, there is very little information of the repeated use of t-PA in patients.