Coronary thrombolysis with intravenously administered human tissue-type plasminogen activator produced by recombinant DNA technology*

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ABSTRACT Coronary thrombolysis was induced by intravenous infusion of human tissue-type plasminogen activator (recombinant human t-PA or rt-PA) obtained by expression of the cloned gene in a mammalian cell system. Thrombolysis was detected by the appearance of reperfusion arrhythmia and confirmed by repeat angiography in anesthetized dogs with 1-hr-old thrombi of the left anterior descending coronary artery that were induced with a copper coil. Infusion of 1000 IU (10 μg/kg/min) intravenous rt-PA (n = 9) elicited reperfusion within 13.7 ± 1.9 min (mean ± SE) without producing systemic fibrinolysis or distal coronary embolization. Infusion of urokinase at the same rate elicited thrombolysis in seven of 10 dogs within an average of 19.3 ± 2.2 min. However, distal coronary embolization occurred in two dogs and systemic fibrinolysis was observed in all. In three dogs treated with urokinase thrombolysis was obtained only with subsequent intracoronary infusion. Restoration of myocardial perfusion and metabolism assessed with positron-emission tomography was consistently noted in dogs treated with rt-PA. Thus, rt-PA, a clot-selective thrombolytic agent that does not activate the fibrinolytic system systemically and that is potentially available in large quantities, in view of its synthesis by recombinant DNA technology, offers a promising practical approach for coronary thrombolysis in patients with acute myocardial infarction.


THROMBOSIS in an atherosclerotic coronary artery occurs in most patients with transmural myocardial infarction at the time of the acute episode. Whether or not the clot initiates the infarction or is an early secondary event, it appears increasingly likely that early thrombolysis may be beneficial. Intracoronary infusion of streptokinase results in recanalization in as many as 80% of patients in this category. Based on the assumption that timely recanalization may result in the salvage of jeopardized myocardial tissue, decreased mortality, and improved quality of life, several large-scale studies of the early treatment of myocardial infarction with streptokinase or urokinase have been undertaken. Unfortunately, however, administration of agents conventionally used, such as streptokinase or urokinase, gives rise to a systemic lytic state, a condition associated with unavoidable risk. Such agents convert plasminogen to plasmin, either directly or indirectly, in the systemic circulation. Plasmin exerts proteolytic activity in the plasma, leading to depletion of fibrinogen and of plasminogen along with consumption of α₂-antiplasmin. Furthermore, the breakdown products of fibrinogen (fibrinogen degradation products or FDPs) accumulate. In concert, these changes impair coagulation and predispose to systemic bleeding, especially in patients such as those with infarction who may require early surgery or other invasive procedures. Because the depletion of fibrinogen is prolonged and because FDPs persist for protracted intervals, the bleeding tendency may be quite persistent. With agents such as streptokinase, which have a longer biological half-life in the circulation than tissue-type plasminogen activator (t-PA), persistence of the bleed-
ing diastasis is particularly prominent. Systemic activation of the fibrinolytic system occurs even when these agents are administered by the intracoronary route because their levels in systemic blood become quite high. Schroeder et al.\textsuperscript{4} have recently demonstrated that systemic infusion of very high doses of streptokinase (1.5 million U/60 min) elicits recanalization with a frequency similar to that obtained after intracoronary administration. This procedure is, however, associated with systemic activation of the fibrinolytic system, which may also give rise to bleeding. Others have reported that streptokinase administered systemically is less effective than that by the intracoronary route.\textsuperscript{5, 6}

We have previously shown\textsuperscript{7} that intravenous administration of t-PA isolated from culture fluid of melanoma cells\textsuperscript{7} results in prompt coronary thrombolysis in dogs without systemic activation of the fibrinolytic system. This agent converts plasminogen to plasmin essentially only when the zymogen is bound to fibrin. Thus, circulating plasminogen is not converted to plasmin, proteolysis does not occur in the systemic circulation, depletion of fibrinogen does not occur, and FDPs do not accumulate with its use.\textsuperscript{8, 9} Purification of the protein that is needed from cell culture fluid is, however, laborious and expensive. Accordingly, acquisition of t-PA by this means is not likely to provide the large amounts of material needed for widespread clinical studies or application.

To overcome these difficulties it would be desirable to produce biologically active t-PA by recombinant DNA technology, which lends itself readily to large-scale production. Pennica et al.\textsuperscript{8} have recently cloned and expressed the human t-PA gene in Escherichia coli. Biological properties of a glycosylated recombinant t-PA (rt-PA) in vitro were found to be very similar to those of the naturally occurring activator obtained from culture fluid of melanoma cells.\textsuperscript{8} Because human rt-PA could constitute a much more practical and readily available agent for clinical research and therapeutic use,\textsuperscript{9} the present study was performed to determine whether rt-PA was effective as a coronary thrombolytic agent in dogs with experimentally induced coronary thrombosis.

**Methods**

In 19 dogs weighing 19 ± 1.5 kg and anesthetized with 10 to 15 mg/kg sodium pentobarbital (n = 13) or with 12.5 mg/kg thiopental and 60 mg/kg a-chloralose (n = 6) coronary thrombosis was induced in each by advancing a 3 to 5 mm long copper coil attached to an intracoronary catheter via the carotid artery 3 to 5 cm into the left anterior descending coronary artery distal to the first main diagonal branch, as previously described.\textsuperscript{9} Occlusion of the thrombus formed within 5 to 10 min and was confirmed angiographically in all dogs. All of the animals studied developed electrocardiographic signs typical of ischemia, but none developed ventricular fibrillation. One to one and one-half hours after induction of coronary thrombus, rt-PA or urokinase was infused intravenously at a rate of 1000 IU/kg body weight/min for 30 min. Complete thrombolysis had not occurred at the completion of infusion in three dogs treated with urokinase. In these animals, the agent was administered subsequently at the same rate but via the intracoronary route for an additional 15 min.

Thrombolysis was generally heralded by the onset of reperfusion arrhythmia and was confirmed in each animal by angiography performed at 10 min intervals. Heparin was administered via intravenous injections of 1000 units at the onset of the infusion of thrombolytic agents and 15 and 30 min later to prevent reocclusion.

For characterization of the extent of systemic activation of the fibrinolytic system blood samples were collected in citrate before and 10, 20, and 30 min after the onset of intravenous infusion, at the end of intracoronary infusion if one was administered, and at 5 min intervals for 20 min after completion of the infusion of thrombolytic agents. Samples were cooled immediately on ice, centrifuged, and frozen at −20° C for subsequent analysis of fibrinogen and a2-antiplasmin activity, as described previously.\textsuperscript{10} Circulating levels of t-PA were measured by immunoradiometric assay as described previously.\textsuperscript{11}

Response of the myocardium to coronary thrombolysis was evaluated in three dogs treated with urokinase and three treated with rt-PA by serial positron-emission tomography before and after thrombolysis. For these studies, myocardial perfusion and metabolic integrity were assessed as previously described.\textsuperscript{12, 13} Briefly, to estimate perfusion, 25 to 36 mCi of \(^{15}C\)-O\(_2\) was administered intravenously and the fraction of labeled water in the myocardium was calculated by blood pool subtraction after inhalation of \(^{15}C\)-O\(_2\) given to label erythrocytes. Myocardial metabolism was assessed after intravenous administration of 20 to 25 mCi \(^{11}C\)-palmitate. Diminished accumulation of radiolabeled palmitate delineates the locus and extent of impairment of myocardial metabolism in this setting. Tomograms were analyzed as described previously.\textsuperscript{6, 13} Tomography was repeated 90

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**TABLE 1**

Comparisons of results obtained with positron-emission tomography in three dogs treated with urokinase (UK) and three treated with rt-PA

<table>
<thead>
<tr>
<th></th>
<th>UK-treated dogs</th>
<th>rt-PA-treated dogs</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Region at risk (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricle</td>
<td>15 ± 13</td>
<td>16 ± 14</td>
</tr>
<tr>
<td>Regional blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% normal</td>
<td>24 ± 18</td>
<td>54 ± 40</td>
</tr>
</tbody>
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Values for the region at risk based on \(^{15}C\)-palmitate accumulation and regional blood flow in the region at risk were measured as previously reported\textsuperscript{6, 12, 13} after intravenous administration of \(^{15}C\)-palmitate and \(^3\)H\(_2\)O. I and II represent results in tomograms before (I) and after (II) administration of thrombolytic therapy. Values are averages ± SD.

Only a small number of dogs were studied tomographically since the primary purpose of this investigation was to determine whether or not rt-PA could elicit coronary thrombolysis and if so whether it conferred metabolic benefit to the heart comparable to that obtained when lysis was induced with other means.
min after the initial study to allow sufficient time for decay of \(^{11}C\) (half-life = 20.4 min) to near background.

Results

rt-PA induced coronary thrombolysis during the course of intravenous infusion in each of the nine dogs studied under this regimen. Reperfusion occurred after 13.7 ± 1.9 min (mean ± SE), and was reflected by reperfusion arrhythmia (ventricular ectopic beats or idioventricular rhythm) and confirmed in each case by angiography (figure 1). Intravenous infusion of urokinase alone resulted in reperfusion in seven of 10 dogs. In two of these dogs angiographically confirmed complete thrombolysis was associated with complete defibrinogenation at the time of clot lysis. In two other dogs angiography revealed peripheral embolization (figure 2). Partial defibrinogenation was evident in both, with a decline of fibrinogen to 55% of the preinfusion value at the time of lysis. In three dogs reperfusion was induced by urokinase only after intracoronary administration had commenced. In these animals it occurred 4, 11, and 19 min after the onset of the intracoronary infusion that followed the intravenous infusion.

Dogs treated with rt-PA exhibited marked recovery of myocardial accumulation of \(^{11}C\)-palmitate (figure 3) in studies in which positron-emission tomography was performed after intravenous administration of the tracer. The favorable tomographic response reflected rapid induction of lysis and considerable recovery of myocardial perfusion (table 1).

Analysis of plasma levels of fibrinogen and \(\alpha_2\)-antiplasmin (figure 4) indicated that infusion of rt-PA did not lead to significant systemic activation of the fibrinolytic system since \(\alpha_2\)-antiplasmin and fibrinogen levels were well maintained. Throughout the interval of the infusion a steady-state level of rt-PA activity in plasma corresponding to activity of approximately 1 \(\mu\)g/ml was maintained. This concentration declined rapidly, however, after the end of the infusion (figure 4).

**FIGURE 1.** Coronary thrombolysis with recombinant t-PA. A, Coronary angiogram before introduction of the copper coil showing normal left coronary artery. B, Coronary angiogram after introduction of the copper coil in the left anterior descending coronary artery showing occlusive thrombosis (indicated by asterisk). C, Coronary angiogram after intravenous infusion of recombinant t-PA showing complete reperfusion and no signs of distal embolization.
4). In contrast, activity of the activator in the systemic circulation persisted after cessation of the infusion of streptokinase or urokinase.

In contrast to the case with rt-PA, infusion of urokinase was associated with systemic activation of the fibrinolytic system. Complete defibrinogenation at the end of the intravenous infusion or partial depletion of fibrinogen at the end of the intravenous or coronary infusion was seen in most dogs exposed to this agent. Fibrinogen decreased to an average of 45% of the preinfusion value (figure 4). The $\alpha_2$-antiplasmin level decreased to less than 40% of the preinfusion value in all dogs studied and became undetectable in four.

**Discussion**

The results of our study indicate that rt-PA is a clot-selective thrombolytic agent with coronary thrombolytic activity comparable to that exhibited by naturally occurring t-PA.\(^6\) In contrast to the case with urokinase, intravenous infusion of rt-PA results in prompt dissolution of coronary thrombi without causing systemic activation of the fibrinolytic system. These results extend our earlier observations with native t-PA and demonstrate that the translation product of the human t-PA gene is effective in eliciting coronary thrombolysis.

rt-PA appears to offer several potential advantages for clinical coronary thrombolysis. Since it can be administered intravenously in high doses without eliciting a bleeding diathesis, the upper bound of dose is not likely to be limited by the risk of bleeding as is the case with streptokinase or urokinase. Since t-PA is a naturally occurring human protein, in contrast to streptokinase, rt-PA is not likely to be antigenic. Hence, antibody-saturating initial doses and paradoxically increased responses to a given dose once the antibody is fully complexed are not likely to be encountered. Furthermore, allergic reactions, encountered not infrequently with streptokinase, are not likely to be a problem. The short biological half-life of rt-PA coupled with the fact that it does not induce a systemic lytic
state allow titration of fibrinolytic activity on an almost minute-by-minute basis, without precluding early implementation of therapeutic options such as coronary artery bypass grafting or percutaneous transluminal angioplasty on the one hand, and permitting maintenance of a sustained fibrinolytic state by continuous infusion on the other. Also in contrast to the case with streptokinase, repeated dosing is not precluded by the

FIGURE 3. Reconstructions from a single midventricular transverse slice obtained with positron-emission tomography before (left) and after (right) intravenous administration of rt-PA in one dog. The perfusion tomograms obtained with H$_2^{15}$O and blood pool subtraction with C$_3^{15}$O (top) show a large defect that resolves after thrombolysis in the region supplied by the left anterior descending coronary artery. The tomograms depicting metabolism reflected by the distribution of $^{11}$C-palmitate (bottom) demonstrate restoration of $^{11}$C-palmitate accumulation in a previously metabolically impaired region after thrombolysis.

FIGURE 4. Changes in some circulating factors involved in the fibrinolytic system during infusion of recombinant t-PA or urokinase in dogs with acute myocardial infarction. C refers only to dogs given rt-PA. $-$ = t-PA (1000 IU/kg/min intravenously for 30 min); ••• = urokinase (1000 IU/kg/min intravenously for 30 min followed by 1000 IU/kg/min of intracoronary drug for 15 min). Results are mean ± SD.
risk of allergic reactions or by anamnestic antibody responses. Since rt-PA is a potentially widely available protein, in view of the fact that it can be synthesized by recombinant DNA technology, this material appears to offer a promising alternative for coronary thrombolysis in patients with acute myocardial infarction.

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