Recombinant tissue plasminogen activator in patients with pulmonary embolism: correlation of fibrinolytic specificity and efficacy

DOUGLAS E. VAUGHAN, M.D., SAMUEL Z. GOLDBERGER, M.D., JAN KIM, B.S., AND JOSEPH LOSCALZO, M.D., PH. D.*

ABSTRACT  Blood samples from 24 patients who received recombinant human tissue-type plasminogen activator (rt-PA) for angiographically documented acute pulmonary embolism were examined to identify and quantify fibrinolysis. Before and after the intravenous administration of 50 mg rt-PA over a 2 hr period, levels of total fibrinogen, fibrin(ogen) degradation products (FDP), and cross-linked fibrin degradation products (XDP) were measured in each patient. Elevated levels of XDP were found in all patients before treatment (mean 2.0 µg/ml, normal < 0.2 µg/ml), and these increased 12-fold with treatment. Fibrinogen levels fell 30% and FDP levels increased 24-fold for the entire group of patients. Over this 2 hr period, 10 of 24 patients (responders) demonstrated 25% or greater improvement in the extent of pulmonary artery thrombus as quantified by Urokinase Pulmonary Embolism Trial score, and these patients were found to have a significantly lower XDP/FDP ratio after rt-PA (p < .04) than those patients who failed to respond. These data suggest that (1) the intravenous administration of pharmacologic doses of rt-PA in patients with pulmonary embolism produces both fibrinolysis and fibrinogenolysis, (2) successful thrombolysis in these patients is associated with a preponderance of fibrinogenolysis over fibrinolysis, (3) the XDP/FDP ratio is a useful indicator of fibrinolytic specificity, and (4) in patients with acute pulmonary embolism the endogenous fibrinolytic pathways are activated, albeit ineffectively, as indicated by the increased circulating XDP levels seen in all 24 patients before the administration of rt-PA.


THE GOAL of thrombolytic therapy is rapid and specific fibrinolysis. While the currently available first-generation thrombolytic agents promptly induce clot dissolution in the majority of patients, the clot specificity of these agents is not optimal. The administration of streptokinase and urokinase predictably produces marked plasma fibrinogen depletion, increases in fibrin(ogen) degradation products (FDP), and the generation of a systemic "lytic" state.¹

Currently, major efforts are underway to develop thrombolytic agents that are not only effective in lysing clots, but are able to do so with minimal systemic fibrinoembolization,²-⁴ thereby lowering the attendant hemorrhagic risk of the administration of such an agent. Recombinant tissue plasminogen activator (rt-PA) was developed with these goals in mind.² However, there is some evidence to suggest that fibrinolysis accompanies, and indeed may be a prerequisite for, successful thrombolysis.⁵ ⁶

During clot formation, the end-to-end and side-to-side linkage of fibrin monomer(s) results in the formation of molecular domains that are antigenically unique to the fibrin molecule. Subsequently, when plasmin-mediated fibrinolysis occurs, unique breakdown products are released that circulate in plasma and can be quantified, including X oligomers and D-D and Y-D dimeric fragments.⁷ ⁸ FDPs, as commonly measured, identify proteolytic derivatives of fibrinogen, fibrin monomer, and cross-linked fibrin, and can be described as nonspecific in nature. Focusing on quantit-
tive changes in cross-linked fibrin degradation products (XDPs) by use of a monoclonal antibody to the D-dimer and comparing these fibrin-specific derivatives to changes in FDPs produces an index of fibrinolytic specificity, the XDP/FDP ratio. An increase in this ratio concomitant with the administration of a thrombolytic agent is indicative of relatively specific fibrinolysis, while a fall in the ratio corresponds to a preponderance of nonspecific fibrinogenolysis. In this study, we have attempted to quantitate and analyze the fibrinolytic effects of an intravenous infusion of rt-PA in 24 patients with angiographically documented pulmonary embolus by measuring XDPs in conjunction with standard measurements of plasma fibrinogen and FDPs. Additionally, we have examined the effect of this thrombolytic agent on the XDP/FDP ratio, particularly in regard to thrombolytic efficacy in this setting.

Methods

The study population included 24 patients 18 years or older who had angiographically documented pulmonary emboli in a segmental or more proximal pulmonary artery within 5 days of the onset of symptoms or signs. The blood samples used in this study were obtained as part of a clinical study designed to determine the safety and efficacy of rt-PA in the treatment of acute pulmonary emboli. Patients were entered into the study after the completion of conventional pulmonary angiography. As soon as acute pulmonary embolism was documented and informed consent was obtained, blood was withdrawn for determination of pretreatment levels of fibrinogen, FDP, and XDPs, after which 50 mg of rt-PA (Activase, supplied by Genentech, Inc.) was infused over 2 hr (25 mg/hr). The first six patients received a formulation containing principally double-chain rt-PA (product code GI11021) and the next 18 patients received a formulation containing principally single-chain rt-PA (product code GI11035). Pulmonary angiography was repeated immediately after the infusion of rt-PA. Importantly, a minimum of 30 min elapsed after the termination of the infusion of rt-PA before blood was drawn for determination of posttreatment levels of fibrinogen, FDPs, and XDPs.

Blood for plasma fibrinogen was drawn into 13 mM citrate and 250 IU/ml of aprotinin (to prevent fibrinogenolysis in vitro) and then immediately placed on ice. Plasma was collected from these samples by centrifugation at 800 g for 15 min; it was stored at −20°C for up to 6 weeks and its fibrinogen content was measured by the sodium sulfite precipitation method of Rampell and Gaffney. FDPs were measured in serum samples according to the method of Mersky et al.11 XDPs were measured in plasma by a semiquantitative latex agglutination method using monoclonal antibodies (Dimertest; supplied by Mabeo, Springwood, Brisbane, Australia) directed against the D-dimer fragment released by plasmin-mediated proteolysis of cross-linked human fibrin.8,12 The lower limit of the assay was 0.2 μg/ml. Serial dilutions were performed on each plasma specimen until agglutination was no longer observed. Individual patient values are reported as the median of the range of the highest observed dilution demonstrating agglutination.

Quantitative assessments of the extent of pulmonary artery thrombus were made by a blinded panel of six investigators, based on the scoring system used in the Urokinase Pulmonary Embolism Trial (UPET).13 Massive pulmonary embolism was defined as a score of 7 to 9, large pulmonary embolism as a score of 3 to 7, and medium pulmonary embolism as a score of less than 3. For purposes of this study, responders were defined as those patients who exhibited a 25% or greater improvement (decrement) in their quantitative score after the 2 hr infusion of 50 mg of rt-PA. Statistical comparisons of data obtained before and after administration of rt-PA were carried out by paired Student’s t test. A p value of >.05 was considered indicative of the lack of a statistically significant difference.

Results

Twenty-four patients were entered into the study, 10 of whom were women. The mean age of the patients studied was 54 years (range 23 to 86). Immediately after the 2 hr infusion of rt-PA, repeat angiography demonstrated significant (as defined in Methods) clot lysis in 10 patients (score 5.9 ± 2.2 before treatment and 2.9 ± 1.6 after treatment, p < .001), and no or minimal lysis in 14 patients (score 7.1 ± 2.3 before treatment and 6.5 ± 2.0 after treatment, p = NS).

Fibrinogen levels fell significantly after the 2 hr infusion of rt-PA (table 1), and this was accompanied by a 24-fold increase in FDP levels. XDP levels, which were elevated in all patients before therapy and ranged from 0.35 to 6 μg/ml (normal ≤0.2 μg/ml), increased 12-fold over the same period of time. Simultaneously, the XDP/FDP ratio, used as an index of fibrinolytic specificity, fell 21%.

Comparisons were made between the hematologic values for the 10 early responders (based on a greater than 25% improvement in quantitative score) and the 14 remaining patients in an attempt to define predictors of clot lysis. Before therapy, there were no significant differences in fibrinogen, FDP, XDP, XDP/FDP, or quantitative score when responders and nonresponders were compared (table 2). After 50 mg of rt-PA, fibrinogen fell by 36% in responders and by 24% in nonresponders, and FDPs increased 32-fold in responders and only 19-fold in nonresponders, but these values failed to reach statistical significance at the p < .05 level. XDPs increased in both groups, but were signifi-

<table>
<thead>
<tr>
<th>TABLE 1 Hematologic variables and quantitative score in all patients (n = 24) before and after treatment with rt-PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Fibrinogen (mg%)</td>
</tr>
<tr>
<td>FDP (μg/ml)</td>
</tr>
<tr>
<td>XDP (μg/ml)</td>
</tr>
<tr>
<td>XDP/FDP</td>
</tr>
<tr>
<td>Score</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD for all patients before treatment and after the infusion of 50 mg rt-PA given over a 2 hr period.
TABLE 2
Comparison of hematologic values and quantitative score for responders and nonresponders before treatment

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Nonresponders</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 14)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg%)</td>
<td>370 ± 125</td>
<td>340 ± 110</td>
<td>NS</td>
</tr>
<tr>
<td>FDP (µg/ml)</td>
<td>6.0 ± 3.0</td>
<td>7.2 ± 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>XDP (µg/ml)</td>
<td>2.1 ± 2.1</td>
<td>1.9 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>XDP/FDP</td>
<td>0.33 ± 0.33</td>
<td>0.56 ± 0.79</td>
<td>NS</td>
</tr>
<tr>
<td>Score</td>
<td>5.9 ± 2.2</td>
<td>7.1 ± 2.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

significantly higher in nonresponders. There was also a statistically significant difference in the posttreatment XDP/FDP ratio in the two groups (table 3): This ratio of fibrinolytic specificity fell a mean of 56% in the responders, as compared with a mean decrease of only 3% in nonresponders (p < .04). As stated in the Methods, all patients received 50 mg of rt-PA over a 2 hour period. This corresponds to a dose of 27.35 ± 1.17 mg/m² in the responders, and a dose of 25.75 ± 2.83 mg/m² in the nonresponders (p = .029).

Discussion

All patients had elevated levels of XDP before therapy, suggesting that endogenous, local fibrinolytic mechanisms are active in patients with acute pulmonary embolism. The 21% decrement we observed (56% in the responders and 3% in the nonresponders) in the XDP/FDP ratio after the administration of rt-PA, coupled with the absolute increase in XDP levels, suggest that exogenous rt-PA, delivered intravenously in the dose and manner we described, induces proteolysis of both fibrin and fibrinogen, but is not as fibrin specific as endogenous fibrinolytic mechanisms.

The administration of rt-PA produced both significant fibrinogenolysis and a disproportionate increase in FDPs over XDPs, particularly in the responders.

TABLE 3
Comparison of hematologic values and quantitative score for responders and nonresponders after treatment

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Nonresponders</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 14)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (mg %)</td>
<td>235 ± 71</td>
<td>257 ± 91</td>
<td>NS</td>
</tr>
<tr>
<td>FDP (µg/ml)</td>
<td>190 ± 151</td>
<td>140 ± 150</td>
<td>NS</td>
</tr>
<tr>
<td>XDP (µg/ml)</td>
<td>17 ± 12</td>
<td>29 ± 16</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>XDP/FDP</td>
<td>0.14 ± 0.09</td>
<td>0.54 ± 0.82</td>
<td>&lt;.04</td>
</tr>
<tr>
<td>Score</td>
<td>2.9 ± 1.6</td>
<td>6.5 ± 2.0</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD for both groups of patients after the administration of 50 mg rt-PA over a 2 hr period.

This apparent loss of fibrin specificity may not be an entirely unfavorable effect, however, since two recent reports have noted that fibrinogenolysis, both with streptokinase and rt-PA, was associated with enhanced thrombolysis efficacy in acute myocardial infarction.5,6 The significantly lower XDP/FDP ratio in responders than in nonresponders in this study adds further credence to this observation, and suggests that some degree of fibrinogenolysis may support or enhance thrombolysis in the setting of acute pulmonary embolism.

That absolute levels of XDPs were significantly greater in nonresponders than responders is a particularly intriguing finding and underscores the complexities involved in both the clinical achievement and laboratory assessment of thrombolysis. It may reflect an inability on our part to quantitate clot size and dissolution adequately with standard angiographic techniques. The quantitation of degree of clot dissolution with the UPET scoring system is well accepted, but does have shortcomings, among which are poorer quantitation of peripheral than central clot and the semi-quantitative and somewhat subjective nature of the angiographic interpretation itself. Many of our patients underwent only unilateral pulmonary angiography, and although it is unlikely, the elevated levels of XDPs in the nonresponders could represent the dissolution of undetected thrombus from the unstudied lung or even from other vascular beds. Alternatively, nonresponders may have had more central clot with smaller surface-to-volume ratios, leading to a need for a relatively larger infusion than that in responders to produce central clot lysis and achieve quantifiable reperfusion.

Interestingly, since the nonresponders received significantly less rt-PA per square meter than did responders, the higher absolute levels of XDPs and higher XDP/FDP ratio in nonresponders suggest that while the administration of lower doses of rt-PA produces greater fibrin selectivity, these doses are not as effective as higher doses at which some of this selectivity is sacrificed for greater efficacy. Our data suggest that some degree of fibrinogenolysis is necessary for the achievement of adequate thrombolysis, as others have suggested in coronary thrombosis.5,6 The mechanism by which this occurs may simply be the inhibition of further fibrin production by adequate concentrations of circulating FDPs, thereby limiting rapid reformation of thrombus at sites of ongoing thrombolysis.

Fourteen of the 24 patients described in this study received additional rt-PA and subsequently underwent further angiographic studies. Among these 14 patients, lysis was ultimately achieved in 10 with the additional...
infusion, supporting the notion that at least some of the nonresponders received an inadequate initial dose of rt-PA when given in this fixed fashion. We chose to analyze the hematologic data after 2 hr of treatment only because it provided the best design in analysis, permitting valid comparisons between groups. All patients received identical treatment for the first 2 hr, except that the first six patients enrolled received an infusion of principally double-chain rt-PA, while the subsequent 18 patients received a formulation of principally single-chain rt-PA. Three of the 10 responders in this study received double-chain material, while three of the 14 nonresponders received the same preparation.

This study represents the initial experience in quantitating XDPs in patients receiving an infusion of a thrombolytic agent for acute pulmonary embolism. The measurement of XDPs in this setting provides a quantitative estimation of the specificity of a fibrinolytic agent. Additional studies and experience with XDPs will hopefully help to refine our knowledge and understanding of fibrinolysis and its relationship with the clinical goal, thrombolysis. Additionally, through the implementation of the XDP/FDP ratio, it is possible to gauge and index noninvasively an agent's fibrinolytic specificity. This ratio may be of value in trials of newer genetically engineered thrombolytic agents and novel dosage regimens. Finally, although fibrinolysis is most certainly a prerequisite for clinically effective thrombolysis, our findings suggest that some degree of fibrinogenolysis may be beneficial as well.

Appendix
Other participating investigators include: Eugene Braunwald, M.D., Patricia Come, M.D., Douglas L. Dawley, M.D., Elliott B. Grossbard, M.D., B. Leonard Holman, M.D., Craig M. Kessler, M.D., Duck Soo Kim, M.D., Gerald M. Kolody, M.D., John E. Markis, M.D., Michael F. Meyerovitz, M.D., J. Anthony Parker, M.D., Ph.D, Arthur Sasahara, M.D., Andrew P. Selwyn, M.D., G. V. R. K. Sharma, M.D., and Sabah Tumech, M.D.

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Recombinant tissue plasminogen activator in patients with pulmonary embolism:
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Circulation. 1987;75:1200-1203
doi: 10.1161/01.CIR.75.6.1200

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