A Randomized Trial of a Single Bolus Dosage Regimen of Recombinant Tissue Plasminogen Activator in Patients with Acute Pulmonary Embolism*

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Experiments in animals have demonstrated that recombinant tissue plasminogen activator (rt-PA) produces continuing thrombolysis after it is cleared from the circulation and that thrombolysis is both increased and accelerated, and bleeding is reduced when rt-PA is administered over a short period. In previous studies in patients with thrombotic disease, rt-PA has been shown to be an effective thrombolytic agent when administered by continuous infusion over a period between 90 minutes and 8 hours. To determine whether a short course regimen of rt-PA can achieve thrombolysis, a double-blind randomized trial has been conducted in which patients with objectively established acute symptomatic pulmonary embolism who were receiving heparin were allocated to either a 2-minute infusion of rt-PA at a dose of 0.6 mg/kg (33 patients) or saline placebo (25 patients). Perfusion lung scanning was used to assess the change in pulmonary perfusion at 24 hours and seven days post-study drug administration. Thirty-four percent of the rt-PA patients had a greater than 50 percent resolution in the perfusion defect at 24 hours compared to 12 percent of placebo patients (p = 0.026). At 24 hours, the mean relative improvement in the perfusion defect was 37.0 percent in rt-PA treated patients compared to 18.8 percent in the placebo group (p = 0.017). By day 7, no difference in lung scan resolution was detected between the groups. There were no major bleeds in either group nor were there any differences in transfusion requirements between groups. Minor bleeding occurred in 15 of the rt-PA patients mainly at angiogram-catheter insertion and venipuncture sites. These results suggest that a bolus regimen of rt-PA produces accelerated thrombolysis and provides an alternative and convenient approach to thrombolytic therapy in patients with pulmonary embolism.

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Treatment of thrombosis by activation of the plasma fibrinolytic enzyme system provides a non-surgical method for relieving pulmonary arterial obstruction. Urokinase and streptokinase, the plasminogen activators presently approved for the treatment of pulmonary embolism, have been shown to induce early lysis of pulmonary emboli when administered over a period of 12 to 24 hours.1-3 These agents are not fibrin-specific and convert circulating plasminogen to plasmin and so produce a systemic lytic state leading to a generalized coagulation defect which could contribute to the increased risk of bleeding.4

Recombinant tissue plasminogen activator (rt-PA), a new plasminogen activator, which preferentially activates plasminogen in the presence of fibrin,5,6 was initially shown to produce effective thrombolysis in experimental animals and because of its relative fibrin-specificity to induce thrombolysis without producing a generalized coagulopathy.7,8

rt-PA has been evaluated in clinical trials in patients with myocardial infarction,9-12 pulmonary embolism,13-15 and venous thrombosis.16 In all of these studies, rt-PA was administered by continuous infusion over a period of between 90 minutes and 8 hours. These regimens are associated with a systemic lytic state, and although they produce less fibrinolysis than streptokinase, for an equivalent thrombolytic effect, they are associated with excessive bleeding.4,11,13

It has been demonstrated that the effectiveness of rt-PA in inducing lysis of experimental venous thrombi and pulmonary emboli in animals is increased by using high concentrations infused over a short interval.17-20 In our own studies, a 15-minute infusion regimen produced less plasma proteolysis and less experimen-
tal bleeding than an identical dose infused over 1 hour or 4 hours.\textsuperscript{17,18} The results of these studies prompted us to undertake a clinical trial to determine whether a bolus injection of rt-PA is effective in producing accelerated thrombolysis in patients with acute pulmonary embolism.

**Methods**

**Patients**

The study population consisted of patients with acute symptomatic pulmonary embolism documented by either pulmonary angiography, or a high probability ventilation-perfusion lung scan (defined as a segmental or greater perfusion defect with ventilation mismatch\textsuperscript{20}), plus deep vein thrombosis confirmed by venography or B-mode ultrasonography. Patients were excluded if they had an active bleeding process, active peptic ulcer disease, familial or acquired bleeding diathesis, or a platelet count <100,000; had a recent (within two months) cerebrovascular accident or other active intracranial process; had recent (less than ten days) major surgery; major trauma, obstetric delivery, or organ biopsy; had severe hypertension (systolic pressure >200 mm Hg, and/or diastolic pressure >100 mm Hg); were pregnant; had clinical symptoms suggestive of pulmonary embolism of more than two weeks’ duration, or had received parenteral heparin for more than 72 hours. Since the efficacy of the experimental bolus regimen was not established in humans, patients with massive pulmonary embolism who were hypotensive and hemodynamically unstable were considered ineligible and if thrombotic therapy was felt to be indicated, they received streptokinase.

The study protocol was reviewed by the institutional review boards of all participating hospitals, and informed consent was obtained from all patients before randomization.

**Recombinant Tissue Plasminogen Activator (rt-PA)**

Predominantly single chain rt-PA (Activase) produced by a recombinant method was obtained from Genentech, South San Francisco, California.*

**Regimens**

All patients received an initial intravenous heparin bolus of 5,000 units followed by heparin by continuous infusion at a starting dose of 30,000 units for the first 24 hours (20,000 units in 500 ml of 5%/5 dextrose/saline solution infused at 31 ml per hour). The heparin dose was adjusted daily according to the results of laboratory monitoring using the activated partial thromboplastin time (APTT) to maintain the results between 55 and 75 seconds (corresponding to approximately 1.5-2 times control using Dade actin-FS PTT reagent).

Patients were assigned according to a prescribed randomized arrangement to treatment with either rt-PA or saline solution placebo. The patients were stratified according to the method of diagnosis (pulmonary angiography or not) and the time since the onset of symptoms of pulmonary embolism (<48 hours or ≥48 hours).

Patients received rt-PA (0.6 mg/kg ideal body weight reconstituted in 50 ml sterile water) by bolus injection over 2 minutes through a side port in the intravenous tubing. The heparin was interrupted only for the duration of the study drug infusion. The same procedure was followed for patients who received the saline solution placebo. The ideal body weight was determined from tables produced by Metropolitan Life 1983. Patients were treated on the general medical ward and were observed closely for evidence of bleeding.

**Follow-up and Outcome Measures**

The effectiveness of the regimens for producing lysis of pulmonary emboli was assessed by comparing the baseline perfusion lung scan with the scan performed at 24 hours and seven days after treatment with rt-PA or placebo. The primary outcome measure, defined before the commencement of the trial, was relative improvement in perfusion of more than 50 percent from the baseline perfusion scan. This outcome was selected because it was considered to represent clinically important resolution of pulmonary perfusion.

In order to determine the size of the perfusion defect, we used a modification of methods previously reported for the UPET study\textsuperscript{29} and by Parker et al.\textsuperscript{30} Patients underwent technetium-99m-labelled macroaggregated albumin perfusion lung scanning consisting of six projections.\textsuperscript{31} Three physicians, experienced in the interpretation of lung scans, reviewed the scans and transposed the area of defect for each view onto a standard coding sheet which portrayed the six views. A perfusion defect was defined as an area of absent perfusion or hypoperfusion. The results of the lung scanning were interpreted without knowledge of the patient’s clinical findings or the treatment group to which the patient had been randomized. Any disagreement in size of the perfusion abnormality was resolved by consensus. The area of the perfusion defect for each view on the coding sheet was calculated by planimetry and the area of defect expressed in cm\textsuperscript{2}.

Specifically, the perimeter of the defect was gated using the Digitpad Digitizer (Houston Instruments, Austin, Texas) and the area was measured using Bioquant System IV Software (R&K Biometrics, Nashville, Tennessee) and an IBM PC microcomputer. The defects for each projection were combined to yield a total perfusion defect for that patient.

In addition, to determine the mean improvement in pulmonary perfusion between pre-treatment and 24 hours and seven days, the change in the total perfusion defect in the anterior and posterior views was used as described in the UPET study.\textsuperscript{29} Patients were examined daily during the ten-day study period for evidence of bleeding. Bleeding was classified as major if it was overt and associated with either a fall in hemoglobin level of 20 g/L or more, or a need for transfusion of two or more units of blood, or if it was retroperitoneal or intracranial. Bleeding was defined as minor if it was overt, but did not meet the other criteria for major bleeding. Daily hemoglobin determinations were performed.

Although mortality and recurrent pulmonary embolism were not primary outcome measures for the trial, they were documented during the first seven days post-randomization.

**Coagulation Studies**

Blood samples were collected prior to rt-PA injection and at 30 and 90 minutes post-injection. At each time point 4.5 ml of blood was collected into a 5 ml Vacutainer tube (BD Vacutainer, Toronto) prefilled with 50 \(\mu\)L of 100 \(\mu\)m/L D-Phe-Pro-Arg-CH\(_2\)Cl (Sigma Chemical, St. Louis) diluted in 10 mmol/L HCl. After careful mixing 4.5 ml of blood was transferred into a second tube containing 0.5 ml of 3.8 percent trisodium citrate. The red cells were sedimented by centrifugation at 1,600 g for 15 minutes at 4°C. The harvested plasma was stored in aliquots at \(-70°C\) until assayed. The plasma fibrinogen concentrations were determined by the method of Clauss.\textsuperscript{32} Alpha-antiplasmin concentrations were measured using the chromogenic substrate CBS 3308 (Diagnostic Stago, Wallmark, Guelph, Ont.).\textsuperscript{33}

**Statistical Analysis**

The proportion of patients in each treatment group who had a 50 percent or more improvement in lung scan was compared using a chi-square test.\textsuperscript{34} Based on the results of the UPET study,\textsuperscript{29,32} it was anticipated prior to commencing our study that treatment with rt-PA would not be inferior to heparin alone in terms of achieving at least a 50 percent improvement in the lung scan and consequently a one-tailed \(p\)-value was used in testing for statistical significance for this outcome. The frequency of bleeding for the two treatment
groups was compared with the chi-square test. The mean improvement in the perfusion defect at 24 hours and seven days was compared between groups using analysis of variance with repeated measures. The data on daily hemoglobin levels, fibrinogen and alpha-antiplasmin were analyzed by analysis of variance with repeated measures.

**Results**

**Patient Population**

Fifty-eight patients with acute pulmonary embolism were randomized to either rt-PA (33 patients) or placebo (25 patients). The presenting symptom in patients who received rt-PA was chest pain in 20 (60.6 percent), dyspnea in 27 (81.8 percent), hemoptysis in five (15 percent), while six (18 percent) had syncope. In the placebo group, chest pain was experienced by 23 (92 percent), dyspnea in 22 (88 percent), hemoptysis in seven (28 percent), and five (20 percent) presented with syncope. Twenty-two (67 percent) of the rt-PA patients had the diagnosis confirmed by pulmonary angiography, six (18 percent) by a high probability lung scan associated with a positive venogram, and five (15 percent) by high probability lung scan associated with positive duplex ultrasonography. In the group who received placebo, 18 (72 percent) had the diagnosis confirmed by pulmonary angiography, four (16 percent) by high probability lung scan associated with a positive venogram, and three (12 percent) by a high probability lung scan associated with positive duplex ultrasonography. The treatment groups were reasonably comparable in terms of baseline characteristics such as age, gender, underlying presence of malignancy, previous history of venous thromboembolic disease, duration of symptoms prior to randomization, and duration of heparin therapy prior to study drug administration (Table 1).

**Perfusion Lung Scan Assessment**

The mean baseline perfusion defect in patients who received rt-PA was 27.4 percent and 21.3 percent in the control patients (Table 1). Repeat lung scans were available for 57 patients (98 percent): 32 in the rt-PA group, and 25 who received placebo. Eleven (34.4 percent) rt-PA patients experienced a greater than 50 percent improvement in the lung scan at 24 hours compared to three (12 percent) placebo patients, p = 0.026. At seven days post-injection, the corresponding figures were 19 (59.4 percent) and 14 (56 percent), respectively. Neither age, gender, presence of cancer, previous venous thromboembolism, duration of symptoms and magnitude of the baseline perfusion defect were significantly different in the rt-PA patients with ≥50 percent improvement in perfusion at 24 hours compared to those with <50 percent improvement. At 24 hours, the mean absolute improvement in the perfusion defect was 9.7 percent in the rt-PA patients compared to 5.2 percent in the placebo group, p = 0.07 and the mean relative improvement in perfusion was 37.0 percent compared to 18.8 percent, p = 0.017 respectively. At seven days, no statistically significant difference was detected for absolute improvement in the perfusion defect, 16 percent in patients treated with rt-PA compared to 11 percent in placebo patients, and mean relative improvement, 58.3 percent compared to 49.4 percent respectively.

**Mortality and Recurrent Pulmonary Embolism**

During the ten-day study period, one patient who received rt-PA died compared to none of the patients in the placebo group. This one patient died 10 hours post-rt-PA injection and at autopsy was found to have a saddle pulmonary embolism. There were no episodes of recurrent pulmonary embolism in either treatment group during the study period.

**Complications of Therapy**

The bolus infusion of the rt-PA was reasonably well tolerated. One patient felt hot and diaphoretic within 10 minutes of the infusion. A second patient felt hot and developed mild hypotension within 2 minutes of the infusion, and a third patient experienced mild hypotension associated with urticaria which resolved within 15 minutes of injection. One patient in the placebo group experienced hypotension shortly after administration of placebo.

There were no major bleeds in either treatment group. (The 95 percent confidence interval on the observed difference of 0 is −13.5 percent to +10.6 percent.) Three patients in each group required transfusions during the ten-day study period. None of these patients had an overt site of hemorrhage and all six patients required transfusion for medical indications unrelated to study drug administration, eg, anemia secondary to chemotherapy. Nine of the rt-PA patients experienced bruising either in the groin at the site of insertion of the pulmonary angiogram catheter and/or at the antecubital fossa venipuncture site which was used for study laboratory tests (Table 2). An additional four patients who received rt-PA had oozing at either

**Table 1—Baseline Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>rt-PA (n = 33)</th>
<th>Placebo (n = 25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.5 ± 2.7</td>
<td>59.6 ± 3.6</td>
<td>0.7</td>
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<tr>
<td>Males/Females</td>
<td>18/15</td>
<td>11/14</td>
<td>0.6</td>
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<tr>
<td>Cancer</td>
<td>5</td>
<td>6</td>
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<tr>
<td>Previous deep vein thrombosis or pulmonary embolism</td>
<td>7</td>
<td>6</td>
<td>0.9</td>
</tr>
<tr>
<td>Duration of symptoms (days)*</td>
<td>5.9 ± 1.0</td>
<td>5.6 ± 0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Duration of heparin therapy (hours)*</td>
<td>42.3 ± 4.0</td>
<td>32.2 ± 3.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Baseline perfusion defect (%)</td>
<td>27.4 ± 3.6</td>
<td>21.3 ± 3.7</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Mean ± SEM.

*Prior to study treatment infusion.
Table 2—Bleeding

<table>
<thead>
<tr>
<th>Type of Bleeding</th>
<th>rt-PA</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bruising</td>
<td></td>
<td></td>
</tr>
<tr>
<td>angiogram site*</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>venipuncture site†</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>both sites‡</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Oozing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>angiogram site</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>venipuncture site</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>both sites</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Serosanguineous oozing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>abdominal wound</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*At site of angiogram catheter-insertion.
†At site of venipuncture in antecubital fossa.
‡At both angiogram catheter-insertion and venipuncture sites.

one or both of these sites. In all cases, the oozing was rapidly and easily controlled with gentle pressure. One patient in each treatment group experienced epistaxis and one patient who received rt-PA had increased serosanguineous drainage from an abdominal wound site. Thus, 15 rt-PA patients (45 percent) experienced minor bleeding compared to one (4 percent) placebo patient, p = 0.0005. There was no significant difference in daily hemoglobin levels between the two treatment groups.

Fibrinogen and Alpha2-Antiplasmin Levels

The fibrinogen levels pre-treatment and at 30 minutes, 90 minutes and 24 hours post-treatment for both treatment groups are presented in Figure 1. For patients who received rt-PA there was an approximately 33 percent decrease in fibrinogen level at 30 minutes. The results of the alpha2-antiplasmin levels are presented in Figure 2. In patients who received rt-PA there was an approximate 50 percent reduction in these levels at 30 minutes.

DISCUSSION

In previous studies of rt-PA in patients with pulmonary embolism, the thrombolytic agent was administered in a dose of between 50 and 100 mg over a period of 2 to 7 hours. These dosage regimens produced a systemic lytic state which was associated with an increased incidence of major bleeding.

In this study, rt-PA was given as a bolus over 2 minutes while patients were fully heparinized, and produced a significant increase in the proportion of patients who achieved greater than a 50 percent improvement in the perfusion defect at 24 hours. In

Mean Fibrinogen Levels Over Time

![Graph showing mean fibrinogen levels over time for rt-PA and placebo patients.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21622/)
addition, rt-PA was associated with a mean absolute improvement in the perfusion defect of 9.7 percent and a mean relative improvement of 37 percent. Although there are obvious limitations in comparing the extent of improvement in pulmonary perfusion between studies, it is interesting to note that the relative improvement in lung scan perfusion in our trial is of the same order of magnitude as reported by Goldhaber et al\textsuperscript{14} and Parker et al,\textsuperscript{23} who gave rt-PA in a higher dose over a longer period of time and noted a 30 percent improvement. Our results are also comparable to the improvement in perfusion reported in the UPET study in which urokinase was given over 12 hours and the mean absolute improvement at 24 hours was 7 percent and the mean relative improvement was 24 percent.\textsuperscript{22} By seven days there was no longer any difference in the resolution seen in patients treated with either rt-PA plus heparin or heparin alone, findings similar to those reported from the UPET trial.

The rationale for choosing the dosage regimen in this study was based on three observations made in animals, namely: 1) that thrombolysis was improved when rt-PA was administered in a high concentration over a short period of time; 2) that rt-PA produced continuing thrombolysis after it was cleared from the circulation; and 3) that bleeding was reduced when the same dose of the drugs was administered over a short period of time.\textsuperscript{17-19} Although a 15-minute infusion was used in our experimental studies and those of Shiffman et al,\textsuperscript{29} the administration of a bolus regimen over 2 minutes was chosen because of its simplicity and convenience.

The bolus infusion regimen of rt-PA was well tolerated but it did cause minor bleeding which was confined to the site of insertion of the angiography catheter or to venipuncture sites and was easily controlled by gentle pressure. Thus, unlike the results in animal experiments, the 2-minute infusion did cause a transient disruption of recently formed hemostatic plugs. In addition, it was associated with a mild plasma proteolytic state which caused a 50 percent fall in alpha\textsubscript{2}-antiplasmin and a 33 percent reduction in fibrinogen. There was no evidence of increased major bleeding. Although the relatively small sample size in our trial may have precluded the detection of a clinically important difference in major bleeding, a so-called type 2 error.

In our study, as in others, lung scanning was used as a surrogate outcome to assess the efficacy of rt-PA treatment. This outcome measure was selected because it is noninvasive and is a reliable index of pulmonary arterial obstruction. This trial was not designed to determine whether the bolus regimen of
rt-PA reduced mortality, an outcome measure which would require a much larger sample size, but to investigate in man the observation in experimental animals that very short infusions of high concentrations of rt-PA accelerate thrombolysis of acute pulmonary embolism with a minimum risk of bleeding.

The optimal regimen for rt-PA has not been established. For patients with massive pulmonary embolism, a regimen that induces very rapid thrombolysis is appealing. Short infusion, high concentration regimens have been reported to induce more rapid thrombolysis than similar doses infused over a longer time interval in experimental pulmonary embolism and in patients with coronary artery thrombosis.

It is possible that even more thrombolysis would be achieved by repeated bolus infusions or by following the initial bolus with a continuous infusion. However, the bolus dose regimen, as used in our study, is effective in achieving accelerated thrombolysis of pulmonary embolism in man, is well tolerated, and is easy to administer to patients with acute pulmonary embolism who are also being treated with heparin. Although the bolus dose regimen does cause disruption of hemostatic plugs at sites of recent vascular invasion, the length of exposure and the period of risk is short. It therefore represents a promising new addition to rt-PA regimens for treating pulmonary embolism.

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Plan to Attend ACCP's

57th Annual Scientific Assembly
San Francisco
November 4-8, 1991