A New Short Infusion Dosage Regimen of Recombinant Tissue Plasminogen Activator in Patients with Venous Thromboembolic Disease*

M. N. Levine, M.D., M.Sc., F.R.C.P.(C);† J. Weitz, M.D., F.R.C.P.(C);‡
A. G. G. Turpie, M.B., Ch.B., F.R.C.P.(C);§
M. Andrew, M.D., F.R.C.P.(C);§ M. Cruickshank, M.D., F.R.C.P.(C); and
J. Hirsh, M.D., F.R.C.P.(C), F.C.C.P.||

Although recombinant tissue-type plasminogen activator (rt-PA) has the potential to induce thrombolysis without producing a generalized coagulopathy, the dosage regimens in present use induce a plasma fibrinolytic state and are associated with bleeding. Animal experiments have demonstrated that rt-PA produces continuing thrombolysis after it is cleared from the circulation and that thrombolysis is increased and bleeding is reduced when rt-PA is administered over a short time period. To determine whether a short course regimen of rt-PA has potential in man, we gave a bolus injection of rt-PA (0.6 mg/kg) concurrently with heparin to five patients with venous thromboembolism. Three patients with angiographically proven pulmonary embolism had marked improvement of the perfusion defects when lung scans were repeated 24 h after rt-PA administration. In one of two patients with thrombosis of the deep veins of the upper extremity the venographic defect resolved completely. In three of four patients there was a mild decrease in fibrinogen and a moderate decrease in α₂-antiplasmin levels. There was no excessive bleeding. These results suggest that a bolus injection regimen of rt-PA has considerable potential in the treatment of thromboembolic disease.

Treatment of thrombosis by activation of the plasma fibrinolytic enzyme system is an attractive concept because by dissolving thrombi it provides a nonsurgical method for relieving vascular occlusion. Two plasminogen activators, urokinase and streptokinase (SK), are presently in clinical use for the treatment of pulmonary embolism (PE).¹ Both convert plasminogen to plasmin in the circulation and produce a systemic lytic state leading to a generalized coagulation defect that contributes to the increased risk of bleeding.¹³⁻¹⁵

Recently a new plasminogen activator, tissue-type plasminogen activator, which preferentially activates plasminogen in the presence of fibrin, has been produced by recombinant DNA technology.⁴⁻⁵ This agent, rt-PA, effectively lyses thrombus in animals and man and, because of its affinity for fibrin, has the potential to induce thrombolysis without producing a generalized coagulopathy.⁶⁻⁷

Recent clinical trials with rt-PA in patients with myocardial infarction,⁶⁻¹¹ PE,¹²⁻¹⁴ and venous thrombosis⁵ have demonstrated that rt-PA is an effective thrombolytic agent. In all of these studies, rt-PA was administered by continuous infusion over a period ranging from 90 min to 8 h.⁶⁻¹⁵ These dosage regimens produce activation of the plasma fibrinolytic system but cause considerably less fibrinogenolysis for equivalent thrombolytic effects than SK.¹⁰⁻¹⁶ Nevertheless, high-dose continuous infusion rt-PA can be associated with excess bleeding.¹⁰⁻¹²

We performed studies in rabbits in which rt-PA produced continuing thrombolysis for over 3 h after it was cleard from the circulation; it was most effective as a thrombolytic agent when infused rapidly.¹⁷⁻¹⁸ The short infusion regimen also produced less plasma proteolysis and less experimental bleeding than an identical dose infused over a 4-h period.¹⁸ These experimental findings raise the possibility that after a rapid infusion, rt-PA binds to fibrin in the thrombus and continues to produce thrombolysis after it has been cleared from the circulation, while a longer infusion augments bleeding because of the continuing presence of circulating rt-PA. The results of these animal experiments and the observation that a continuous infusion of rt-PA produces plasma proteolysis and is associated with a risk of bleeding in man prompted us to perform a pilot study to determine the feasibility of using a bolus injection regimen of rt-PA in patients with venous thromboembolic disease.

Materials and Methods

Subjects

Three consecutive patients with angiographically proven PE and two patients with venographically confirmed axillary or subclavian vein thrombosis were studied. The study protocol was reviewed by
the Institutional Review Board of the Hamilton Civic Hospitals, and signed informed consent was obtained from all patients.

**Regimens**

Predominantly single-chain rt-PA (Activase) produced by a recombinant method was used (Genentech, South San Francisco, CA). Patients received an initial intravenous (IV) heparin bolus of 5,000 U followed by heparin given by continuous infusion at a starting dose of 30,000 U for the first 24 h (20,000 U in 500 ml of 2:1 dextrose/saline infused at 31 ml/h). The heparin dose was adjusted daily according to the results of laboratory monitoring, with the activated partial thromboplastin time (APTT) used to maintain the results between 55 and 75 s (corresponding to approximately 1.5 to 2 times control using Dade actin-FS PTT reagent).

Patients received rt-PA (0.6 mg/kg ideal body weight reconstituted in 50 ml of sterile water) by bolus injection over 2 min through a side port in the IV tubing. The heparin infusion was briefly interrupted during this period. Patients were treated on the general medical ward and were observed closely for evidence of bleeding.

**Follow-up**

In patients with PE, the ventilation-perfusion lung scans were repeated 24 h and seven days after injection. Lung scans were interpreted using the criteria from the UPET study. Patients with upper extremity vein thrombosis underwent repeat venography 24 h after rt-PA injection.

**Coagulation Studies**

Blood samples were collected before rt-PA injection and at 30 and 90 min after infusion. At each time point 4.5 ml of blood was collected into a 5-ml Vacutainer tube (BD Vacutainer, Toronto) prefilled with 50 μl of 100 μmol/L Phe-Pro-Arg-CH,Cl (Sigma Chemical Co, St Louis, Mo). After careful mixing, 4.5 ml of blood was transferred into a tube containing 0.5 ml of 3.8 percent trisodium citrate. The red cells were sedimented by centrifugation at 1,600 g for 15 min at 4°C. The harvested plasma was stored at -70°C until assayed. The plasma fibrinogen concentrations were determined by the method of Clauss. α-Antiplasmin concentrations were measured using the chromogenic substrate CBS 3308 (Dade actin-FS PTT reagent).

**RESULTS**

Three patients with angiographically confirmed PE received rt-PA (40, 39, and 50 mg, respectively). The 24-h perfusion lung scans showed marked improvement in all three patients. In one patient there was a 90 percent improvement in perfusion 24 h after rt-PA administration (Fig 1) and complete resolution by seven days. The remaining two patients experienced 50 percent to 75 percent improvement by 24 h, with no further improvement at seven days.

Two patients with upper extremity vein thrombosis received rt-PA; one had thrombosis of the axillary and subclavian vein while the other had subclavian vein thrombosis. The first patient showed no improvement on the second venogram, but the second patient had complete resolution of the defect at 24 h and marked resolution of clinical symptoms. The bolus rt-PA regimen was well tolerated in all five patients and there was no significant hemorrhage. One patient had slight oozing from a venipuncture site approximately 30 min after administration of the rt-PA bolus. However, the oozing lasted less than 1 min and was readily controlled with gentle pressure.

In three of four patients there was a slight decrease
in fibrinogen and a moderate decrease in α2-antiplasmin concentrations. In the fourth patient there was a marked fall in the fibrinogen level (see Table 1). Repeated blood samples could not be obtained from the fifth patient because of poor venous access as the result of systemic combination chemotherapy. There was no decrease in the hemoglobin concentration following treatment in any of the patients.

**DISCUSSION**

rt-PA is a promising new thrombolytic agent that has been shown to be highly effective in inducing lysis of coronary artery thrombi, PE, and venous thrombi. The optimal dosage regimen for rt-PA has not been established. The dosage regimens in current use induce a plasma fibrinolytic state and can be associated with an increased rate of bleeding.

The mechanism of bleeding caused by fibrinolytic agents is complex and likely to be contributed to both by plasmin-mediated lysis of fibrin in hemostatic plugs and by the induction of a generalized coagulation abnormality characterized by hypofibrinogenemia, elevated levels of fibrin degradation products, and reduction of other coagulation factors. It is also possible that the hyperplasminemic state produces a platelet function defect due to hydrolysis of platelet membrane glycoproteins that participate in the aggregation reaction.

Our choice of dosage regimen was based on observations in animals that thrombolysis is improved and bleeding reduced with short-course treatment and that rt-PA produces thrombolysis after it is cleared from the circulation; we also considered that the risk of bleeding might be reduced by minimizing plasma proteolysis and reducing the time that rt-PA remains in the bloodstream. The possibility that the incidence of bleeding might be reduced by shortening the duration of rt-PA infusion is supported by two recent clinical studies. In the first, patients with unstable angina were randomized to either rt-PA infused over 12 h or placebo. Bleeding occurred in eight of 11 patients who received rt-PA, and all of the episodes occurred toward the end of the 12-h infusion. In the second study, Owen et al compared the plasma proteolytic effects of thrombolytic doses of rt-PA and streptokinase in the TIMI study; the fibrinolytic effect produced by rt-PA was delayed and less marked than with streptokinase.

In our study, rt-PA was given as a bolus while the five patients were fully heparinized and was associated with marked thrombolysis in four of five patients; in two the improvement was dramatic. Although there was mild plasma proteolysis in three of the four patients in whom the tests were performed, there was no excessive bleeding. In one patient the fibrinogen and α2-antiplasmin concentrations remained decreased for more than 48 h after rt-PA administration, suggesting that in this patient there was either a defect in the hepatic synthesis of these proteins or impaired clearance of rt-PA.

The interpretation of our findings is limited by the small patient sample and the lack of a control group. Nevertheless, the findings appear sufficiently promising to warrant further exploration of the use of a bolus dose of rt-PA. From previously published reports, it is unlikely that the marked resolution in four of the five patients would have occurred with heparin alone. We are sufficiently encouraged by the results of the pilot study and have commenced a double-blind randomized trial of bolus rt-PA plus heparin vs heparin alone in patients with PE. If the findings of the randomized trial confirm the results of the pilot study, they would represent an important advance in the use of rt-PA as a thrombolytic agent. The bolus injection is simple and likely to be less expensive than continuous infusion, it can be given without interrupting heparin therapy, and it does not require laboratory monitoring.

**REFERENCES**

2 Urokinase Pulmonary Embolism Trial. Phase I results. JAMA 1970; 214:2163-72
3 Urokinase-Streptokinase Embolism Trial. Phase II results. JAMA 1974; 229:1606-13
6 Hoylaerts M, Rijken DC, Lihnen HR, Collen D. Kinetics of rt-PA in the circulation. JAMA 1984; 251:2163-72

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fibrinogen (g/L) Pre</th>
<th>30 min</th>
<th>90 min</th>
<th>α2-Antiplasmin (U/ml) Pre</th>
<th>30 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>4.2</td>
<td>3.9</td>
<td>3.5</td>
<td>1.0</td>
<td>0.47</td>
<td>0.55</td>
</tr>
<tr>
<td>AF</td>
<td>4.3</td>
<td>0.75</td>
<td>0.72</td>
<td>1.29</td>
<td>0.34</td>
<td>0.37</td>
</tr>
<tr>
<td>JG</td>
<td>4.8</td>
<td>3.2</td>
<td>3.5</td>
<td>0.99</td>
<td>0.48</td>
<td>0.56</td>
</tr>
<tr>
<td>MG</td>
<td>4.3</td>
<td>3.8</td>
<td>4.2</td>
<td>1.6</td>
<td>0.47</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*Patients AT and MG had thrombosis of deep veins of an upper extremity; patients AF and JG had PE.*

Table 1—Coagulation Studies

**New Short Infusion Dosage Regimen for rt-PA (Lavine et al)**
the activation of plasminogen by human tissue plasminogen activator. J Biol Chem 1982; 257:2912-19
7 Collen D. Tissue-type plasminogen activator: therapeutic potential in thrombotic disease states. Drugs 1986; 31:1-5
20 van Claus A. Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. Acta Haematol (Basel) 1957; 17:237
22 Adelman B, Michelson AD, Greenberg J, Handin RI. Proteolysis of platelet glycoprotein Ib by plasmin is facilitated by plasmin lysine-binding regions. Blood 1996; 88:1290-84