Recombinant Tissue-Type Plasminogen Activator in Canine Embolic Pulmonary Hypertension

Effects of Bolus Versus Short-term Administration on Dynamics of Thrombolysis and on Pulmonary Vascular Pressure-Flow Characteristics

Richard M. Prewitt, MD, FRCP(C), Frank Shiffman, PhD, David Greenberg, MD, Robert Cook, MSc, and John Ducas, MD, FRCP(C)

We used a canine model of embolic pulmonary hypertension, induced by injection of autologous radioactive blood clots, to investigate effects of recombinant tissue-type plasminogen activator (rt-PA) on dynamics of thrombolysis and on pulmonary pressure-flow (PQ) characteristics. Over 5 (rt-PA₅) or 15 (rt-PA₁₅) minutes, 1 mg/kg rt-PA was infused. Rate and extent of thrombolysis were assessed by counting over both lung fields with a gamma camera. Emboli increased mean pulmonary artery pressure from 14 to 36 mm Hg (p < 0.005). This change was predominantly due to an increase in the effective outflow pressure (Pₑ) from 9 to 29 mm Hg, p < 0.001, obtained by extrapolation from the linear PQ relation. While pulmonary hemodynamics improved with rt-PA₅ and rt-PA₁₅, the change was greatest with rt-PA₁₅. For example, the increase in Pₑ that occurred with embolization was abolished with rt-PA₁₅. Also, the decrease in pulmonary artery pressure was greatest with rt-PA₁₅. While not significantly different, extent of total clot lysis tended to be greatest with rt-PA₁₅ (p < 0.07). Also, while during infusion, the concentration of rt-PA₅ was threefold that of rt-PA₁₅, the corresponding rate of thrombolysis was similar with rt-PA₅ and rt-PA₁₅. These results indicate that the improvement in pulmonary hemodynamics with rt-PA is primarily explained by a decrease in Pₑ. Furthermore, they suggest an upper limit to the dose–thrombolytic rate relation with rt-PA. (Circulation 1989;79:929–938)

Massive pulmonary embolism may result in right ventricular failure, cardiogenic shock, and death.¹⁻⁴ While thrombolytic therapy has been demonstrated to accelerate resolution of emboli and improve hemodynamic status,⁴ few studies have prospectively investigated thrombolytic efficacy of different dosing regimens.

A recent study, using a canine model of pulmonary hypertension induced by injection of radioactive autologous blood clots, compared efficacy of 1 mg/kg recombinant tissue-type plasminogen activator (rt-PA) given over 15 minutes (rt-PA₁₅) and over 90 minutes (rt-PA₉₀).⁵ Initial rate of thrombolysis and decrease in pulmonary artery pressure (PAP) was greatest with rt-PA₁₅.

The major objective of the present study is to investigate the effects of rt-PA on pulmonary vascular pressure-flow (PQ) characteristics. Because of untested assumptions inherent in the conventional pulmonary vascular resistance (PVR) equation,⁶ ⁷ several recent studies have used the PQ relation to investigate pathophysiologic and treatment of pulmonary hypertension.⁸⁻¹² Over physiologic flow rates, this relation is consistently reported as linear. Accordingly, two variables are used to describe pulmonary hemodynamics. First, the slope of the PQ relation defines the pressure change per unit change in flow. When plotted with pressure as the dependent variable, this relation describes the incremental vascular resistance (IR). Second, the extrapolated pressure intercept of the PQ relation (Pₑ) may define the effective outflow pressure of the pulmonary vasculature.⁸ ¹³ Figure 1 illustrates a repre-
sentative pulmonary vascular PQ relation before embolization. Note that the PQ coordinates are well described by a straight line. In this example, the extrapolated pressure intercept (P0) is 8 mm Hg and the slope (IR) is 2 mm Hg/l/min.

Previous work in canine embolic pulmonary hypertension has documented that while both IR and P0 increase with embolization, the increase in PAP is predominantly due to an increase in P0. Accordingly, this study is designed to test the hypothesis that the improved pulmonary hemodynamics with rt-PA are explained by a decrease in P0.

As cited above, a recent study of embolic pulmonary hypertension demonstrated increased rate of clot lysis with rt-PA15 versus rt-PA90. To extend this work, the present study also tests the hypothesis that if the same total dose of rt-PA is given over 5 (rt-PA15) versus 15 (rt-PA90) minutes, the initial rate of thrombolysis and improvement in PAP would be most rapid with the former regimen.

Methods

Thirteen dogs (18.5-27.5 kg) were anesthetized with intravenous pentobarbital (30 mg/kg) and supplemented as required to maintain apnea. Each dog was mechanically ventilated in the supine position via endotracheal tube with 100% O2 at a tidal volume of 20 ml/kg. Rate was adjusted to maintain PaCO2 between 25 and 45 mm Hg. Metabolic acidosis was treated as required with sodium bicarbonate to maintain arterial pH greater than 7.28. A catheter was inserted into the right brachial artery. Measurements of blood pressure were obtained from this catheter and 200 ml blood was drawn for formation of autologous clot. An intravenous line was inserted into the right brachial vein for volume infusion. A thermistor-tipped, flow directed Swan-Ganz catheter (Electro Catheter Corporation, Rahway, New Jersey) was inserted via the external jugular vein and positioned in the proximal pulmonary artery. Measurements of cardiac output (CO), mean PAP, and pulmonary capillary wedge pressure (PCWP) (when possible) were obtained from this catheter. A second Swan-Ganz catheter was placed in the right ventricle to obtain measurements of right ventricular (RV) pressure. When possible, a pigtail catheter (Cordis Corporation, Miami, Florida) was inserted via the left carotid artery into the left ventricle for measurement of left ventricular end-diastolic pressure (LVEDP). Each catheter was connected to a Statham P23 transducer (Gould, Oxnard, California) leveled to midchest. Transducer output was displayed on a 12-channel Electronics for Medicine oscillograph (PPG, Biomedical Systems, Pleasantville, New York). A third Swan-Ganz catheter was positioned in the right atrium for injection of saline bolus for determination of CO by computer (Columbus Instruments, Columbus, Ohio). Plastic fistulas with tapered ends were inserted at three positions to directly connect the arterial and venous circulation and thus to regulate blood flow. The fistulas were inserted between each femoral artery and its corresponding vein and between the right carotid artery and right jugular vein. To obtain multiple PQ coordinates, in each condition, the flow rates through the fistulas were regulated by adjust-
able metal clamps. The fistula in the right leg was modified by the addition of two rubber arms, one for injection of radioactive clot and the other for injection of saline flush. Figure 2 illustrates the experimental preparation. To attempt to minimize bleeding from incision sites, thrombostat (Parke-Davis Canada, Scarborough, Ontario, Canada) at a concentration of 1,000 units/ml isotonic saline was applied.

Radioactive Autologous Blood Clot Formation

A low specific activity 99mTc sulfur colloid preparation was created by boiling 3.0 ml 1N HCL, 3.0 ml Na2S2O3·5H2O and 1.0–1.5 GBq (1GBq = 27 mCi) of 99mTc pertechnetate in 9.0 ml saline for 3.5 minutes. 99mTc sulfur colloid (TSC) was chosen to label clot because of its known affinity for fibrin strands and because the small particles (0.1 μm) when released are rapidly cleared by the reticuloendothelial system (serum t½, approximately 2 minutes), making background correction unnecessary.14,15 After ice bath cooling for 5 minutes, 0.3 ml human serum albumin and 8.0 ml phosphate buffer were added. High quality preparations (98.4 ± 0.3%) were confirmed using instant thin layer chromatography in methyl ethyl ketone.

Autologous clot was formed by slowly dripping 100 ml freshly drawn unheparinized dog blood with 7.0 ml (350 MBq) of TSC and 10,000 units (10 ml) thrombin into a shielded 500 ml Pyrex beaker. The mixture was allowed to stand for 2 hours until the clot had a "Jello-like" consistency. The serum was decanted and discarded. The low specific activity TSC ensured a large number of particles with adequate distribution in the clot matrix. The use of a small volume of TSC and the glass-walled container were necessary to develop a solid thrombus. The clot was then cut into approximately 1-ml aliquots and loaded into 60-ml syringes before injection.

Protocol

After obtaining baseline measurements including multiple PQ coordinates, autologous clot was injected over a 30-minute period via the modified fistula. Each clot injection was followed by saline flush. Initially, clot was injected to increase the PAP to approximately 55 mm Hg. Over the next 20 minutes, when the PAP dropped below 45 mm Hg, additional clot was injected to increase the pressure to approximately 55 mm Hg. Subsequently, the preparation was allowed to stabilize for approximately 45 minutes. Hemodynamic measurements were obtained at 10–15-minute intervals to ensure stability, defined as less than a 10% change in CO and PAP over two consecutive measurements 10 minutes apart. After stabilization, the CO and, thus, PAP were manipulated by sequentially opening the fistulas to create five to six PQ coordinates. During generation of the PQ coordinates, LV filling pressure (PCWP or LVEDP) varied less than 2 mm Hg. The dogs were then randomly selected for intrave-
nous treatment with rt-PA (Genentech, Toronto, Ontario, Canada) at 1.0 mg/kg infused over 5 or 15 minutes. At the beginning of the experiment, 12 slips of paper, each specifying one of the treatment groups, were placed in a container. At the point of randomization, one of the treatments was chosen by blindly selecting one of these slips. An extra rt-PA15 slip was later added for randomization after results for one dog were inadvertently erased from the computer. Therefore, hemodynamic results were available for an extra dog in the rt-PA15 group. Each treatment was followed by a heparin bolus of 100 units/kg and heparin infusion at 10 IU/kg/hr for the remainder of the experiment. Infusion of hetastarch at 100 ml/hr was started at the beginning of rt-PA infusion and was continued until the conclusion of the experiment.

General hemodynamic measurements (BP, PAP, CO, RVEDP, and LVEDP or PCWP) were obtained at the following times: baseline, before clot injection; after embozation, immediately before treatment; and at 15, 30, 45, and 60 minutes and at 1½, 2, 2½, and 3 hours after onset of drug infusion. General hemodynamic results were obtained for seven rt-PA15 dogs and six rt-PA5 dogs.

Assessment of Pulmonary Thrombolysis

Monitoring of chest activity was achieved with a Picker Dyna IV mobile gamma counter (Picker International Canada, Winnipeg, Manitoba, Canada) with a parallel hole collimator coupled to a Medical Data Systems A2 mobile computer (Medtronic of Canada, Richmond, British Columbia, Canada). Four hour dynamic acquisition was in a 64 × 64 matrix at a rate of 60 sec/frame. Thus, an image was obtained each minute over the course of the experiment. Regions of interest were placed about the lung fields. To assess total pulmonary thrombolysis, counts in the lungs were summed over the 10 minutes just before administration of therapy and were compared with a decay-corrected image from the final 10 minutes. To assess rate of pulmonary thrombolysis, the pulmonary time-activity curves were normalized to the maximum counts in the lung fields, which always occurred near the end of the clot infusion. Subsequently, a marker was placed at the onset and end of drug infusion, and a linear best-fit curve between these coordinates was generated by the computer. Thus, the slope of the time-activity curve defined the rate of clot lysis during drug infusion. This relation is expressed in terms of the percent decline of total lung counts per hour.

Data Analysis

The total clot lysis and the rate of clot lysis during infusion were compared between groups by an unpaired t test.

Hemodynamic parameters were analyzed for a change with embolization by a paired t test and for a change with time after onset of treatment by a two-way ANOVA. These hemodynamic measurements, plus the change in PAP from infusion onset, were compared between groups at each time interval by an unpaired t test. To assess effects of embolization and rt-PA on PQ relations, in each condition, PQ coordinates were analyzed by linear regression analysis (least-squares method). They were considered to fit a linear relation at p < 0.05. CO was the independent variable and PAP the dependent variable. The slopes (IR) and extrapolated intercepts (PI) of the pressure-flow lines were compared between groups by an unpaired t test. A change over time in either parameter within a group was tested by a two-way ANOVA. If any two-way ANOVA was significant (p < 0.05), a Student’s-Newman-Keuls test was used to determine which points were different.

Results

Table 1 illustrates mean hemodynamic values before and after embolization for all 13 dogs. Embolization caused a marked increased in PAP (p < 0.0005) and in RVEDP (p < 0.05) and a decrease in mean CO. Since CO increased in two of the rt-PA5 dogs after embolization, the decrease in CO was only significant in dogs subsequently given rt-PA15. BP decreased in dogs subsequently given rt-PA15 (p < 0.025) and remained constant in the group that was subsequently treated with rt-PA5. LV filling pressure remained constant with embolization.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Condition</th>
<th>BP (mm Hg)</th>
<th>PAP (mm Hg)</th>
<th>CO (l/min)</th>
<th>RVEDP (mm Hg)</th>
<th>LV filling pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rt-PA5</td>
<td>Preembolization</td>
<td>148±8</td>
<td>14.3±1.2</td>
<td>2.9±0.4</td>
<td>3.9±1.0</td>
<td>6.8±1.7</td>
</tr>
<tr>
<td>(n=6)</td>
<td>Postembolization</td>
<td>137±8</td>
<td>35.5±2.2*</td>
<td>2.7±0.4</td>
<td>6.9±1.0†</td>
<td>8.5±2.5</td>
</tr>
<tr>
<td>rt-PA15</td>
<td>Preembolization</td>
<td>156±10</td>
<td>14.1±0.9</td>
<td>3.2±0.6</td>
<td>3.2±1.0</td>
<td>7.5±1.2</td>
</tr>
<tr>
<td>(n=7)</td>
<td>Postembolization</td>
<td>133±8‡</td>
<td>35.7±2.1*</td>
<td>2.0±0.4‡</td>
<td>5.8±1.0§</td>
<td>7.3±1.8</td>
</tr>
</tbody>
</table>

BP, blood pressure; PAP, pulmonary artery pressure; CO, cardiac output; RVEDP, right ventricular end-diastolic pressure; LV, left ventricular; rt-PA5, rt-PA15, recombinant tissue-type plasminogen activator infused over 5 or 15 minutes, respectively.

*p<0.0005 when compared with preembolization.

†p<0.01 when compared with preembolization.

‡p<0.025 when compared with preembolization.

§p<0.05 when compared with preembolization.
Table 2. Effects of rt-PA5 and rt-PA15 on Rate of Clot Lysis During Infusion

<table>
<thead>
<tr>
<th>rt-PA5 % Clot lysis/hr</th>
<th>rt-PA15 % Clot lysis/hr</th>
<th>r</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>0.99</td>
<td>72</td>
<td>0.996</td>
</tr>
<tr>
<td>72</td>
<td>0.999</td>
<td>43</td>
<td>0.970</td>
</tr>
<tr>
<td>65</td>
<td>0.998</td>
<td>36</td>
<td>0.990</td>
</tr>
<tr>
<td>76</td>
<td>0.989</td>
<td>32</td>
<td>0.970</td>
</tr>
<tr>
<td>35</td>
<td>0.987</td>
<td>68</td>
<td>0.990</td>
</tr>
<tr>
<td>54</td>
<td>0.995</td>
<td>61</td>
<td>0.995</td>
</tr>
</tbody>
</table>

Mean±SEM 58±6 0.993±0.002 52±7 0.985±0.005

Table 3. Effects of rt-PA5 and rt-PA15 on Percent Total Clot Lysis Over a 3-Hour Period

<table>
<thead>
<tr>
<th>rt-PA5</th>
<th>rt-PA15</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>51</td>
</tr>
<tr>
<td>47</td>
<td>38</td>
</tr>
<tr>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>23</td>
<td>51</td>
</tr>
<tr>
<td>27</td>
<td>51</td>
</tr>
</tbody>
</table>

Mean±SEM 36±4 45±3

Table 2 illustrates individual and mean effects of rt-PA5 and rt-PA15 on rate of clot lysis. Despite a threefold increase in rate of infusion with rt-PA5 compared with rt-PA15, rate of thrombolysis during drug infusion (5 minutes for rt-PA5 and 15 minutes for rt-PA15) was similar. Also, as illustrated in Figure 3, rate of lysis during infusion was relatively constant. The count-time coordinates, obtained during drug infusion, were well described by linear regression analysis (r) and are also depicted in Table 2. An exponential equation did not significantly improve the description of these data. As illustrated in Figure 3, after drug infusion, the rate of lysis rapidly attenuated and over the 3rd hour was relatively constant with both regimens. Table 3 depicts individual and mean values for total clot lysis over 3 hours. As illustrated, while not significant at p<0.05, there was a trend favoring total clot lysis with rt-PA15 (p<0.07). Figure 4 plots mean±SEM values and illustrates hemodynamic effects of embolization and rt-PA on pulmonary hemodynamics and RVEDP. Note the marked increase in PAP with embolization and the subsequent decrease with rt-PA (Panel A). Compared with postembolization, both rt-PA5 and rt-PA15 significantly decreased PAP. While both rt-PA regimens decreased PAP, the change tended to be greater with rt-PA15 and, comparing between groups, at 90 minutes, PAP was significantly lower with rt-PA15. Panel B plots the relative decrease or change in PAP with rt-PA5 and rt-PA15. As with absolute PAP, the average decrease in PAP was consistently greatest with rt-PA15. As illustrated, from 45 minutes to 2½ hours, this change was significantly greater with rt-PA15 than with rt-PA5.

As depicted in Panel C, in both groups, RVEDP significantly increased with embolization and significantly decreased with rt-PA. After 5 minutes of rt-PA5 treatment, the RVEDP was significantly lowered compared with postembolization and remained lower for the remainder of the experiment (p<0.001—p<0.005). Similarly, with rt-PA15, RVEDP was lowered compared with the postembolization condition, from 45 minutes to 3 hours (p<0.025—p<0.001). While, from 90 minutes to 3 hours, mean values tended to be lower with rt-PA15 than with rt-PA5, these differences were not significant at any time.

Effects of embolization and treatment on CO are depicted in Panel D. Note that the decrease in CO with embolization was only significant in dogs that were subsequently treated with rt-PA15 (p<0.01). After embolization, CO and the change in CO were similar within and between groups over time.

Values for LV filling pressure (PCWP or LVEDP) were within the normal range and remained similar within and between groups over time. Also, values for BP were similar between groups over time.

As described in “Methods,” for each dog linear regression analysis was performed on the PAP-CO coordinates before and after embolization and at hourly intervals for 3 hours after rt-PA. All PAP-CO relations were well described by linear regression analysis (i.e., all were significant to at least p<0.05 and the great majority were significant at p<0.01). Mean±SEM values for slope (IR) and extrapolated pressure intercept (P1) in each of these conditions are displayed in Table 4. Note the marked increase in P1 with embolization (p<0.0005). The mean value for IR increased. However, this change was only significant for dogs subsequently treated with rt-PA15 (p<0.025). Most significantly, while IR
remained constant in both groups, there was a marked decrease in $P_t$ with rt-PA. Corresponding to the larger decrease in PAP with rt-PA$_{15}$, the improvement in $P_t$ was greatest. Comparing within groups, note that with rt-PA$_{15}$, values for $P_t$ at 1, 2, and 3 hours were similar to control. That is, by 1 hour rt-PA$_{15}$ totally abolished the marked increase in $P_t$ that occurred with embolization. In contrast, with rt-PA$_5$, $P_t$ was higher than control at 1 hour. Also, comparing between groups, compared with postembolization, the change in $P_t$ was greatest with rt-PA$_{15}$ at 1 and 3 hours ($p<0.01$ and $p<0.05$, respectively).

Figure 5 illustrates effects of embolization and both rt-PA regimens on PQ characteristics in representative dogs. Note the marked increase in $P_t$ and the smaller change in IR with embolization. Further, note the marked decrease in $P_t$ at 1, 2, and 3 hours.

All incision sites for catheter placement were left open, and there was no obvious difference in bleeding between groups. Also, hematocrit was mea-

**TABLE 4. Effects of Embolization and Treatment With rt-PA$_5$ and rt-PA$_{15}$ on the Slope (IR) and Extrapolated Pressure Intercept ($P_t$) of Pressure-Flow Lines**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Condition</th>
<th>IR (mm Hg/l/min)</th>
<th>$P_t$ (mm Hg)</th>
<th>% change in $P_t$ after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>rt-PA$_5$</td>
<td>Baseline</td>
<td>2.1±0.4</td>
<td>8.7±1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Postemboli</td>
<td>3.5±0.4</td>
<td>28.2±2.4*</td>
<td></td>
</tr>
<tr>
<td>(n=6)</td>
<td>1 hr</td>
<td>3.1±0.4</td>
<td>14.3±2.2†</td>
<td>49.7±5.5</td>
</tr>
<tr>
<td></td>
<td>2 hr</td>
<td>3.4±0.4</td>
<td>12.8±2.4</td>
<td>55.8±6.1</td>
</tr>
<tr>
<td></td>
<td>3 hr</td>
<td>3.3±0.4</td>
<td>12.6±1.9</td>
<td>56.2±3.5</td>
</tr>
<tr>
<td>rt-PA$_{15}$</td>
<td>Baseline</td>
<td>2.0±0.4</td>
<td>7.6±0.8</td>
<td></td>
</tr>
<tr>
<td>(n=7)</td>
<td>Postemboli</td>
<td>4.3±0.4†</td>
<td>29.6±2.6*</td>
<td></td>
</tr>
<tr>
<td>1 hr</td>
<td>3.8±0.4</td>
<td>9.6±1.4</td>
<td>68.4±2.6§</td>
<td></td>
</tr>
<tr>
<td>2 hr</td>
<td>4.6±0.4</td>
<td>9.8±0.7</td>
<td>66.2±2.0§</td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>4.3±0.4</td>
<td>9.7±1.1</td>
<td>66.9±3.1§</td>
<td></td>
</tr>
</tbody>
</table>

$^p<0.001$ when compared with all other times.  
$^†p<0.025$ when compared with baseline.  
$^‡p<0.01$ when compared with rt-PA$_5$.  
$^§p<0.05$ when compared with rt-PA$_5$.  

**FIGURE 4. Plots of mean (±SEM) hemodynamic effects of rt-PA$_5$ and rt-PA$_{15}$.** Panel A: Effects of emboli and treatment on pulmonary artery pressure (PAP) (p<0.05 compared with rt-PA$_5$). Panel B: Effects of rt-PA on the change in PAP postclot (PC) (b and c=p<0.02 and p<0.01, respectively, compared with rt-PA$_5$). Panels C and D: Effects of rt-PA$_5$ and rt-PA$_{15}$ on right ventricular end-diastolic pressure (RVEDP) and cardiac output (CO), respectively. For discussion, see text.
sured at constant times in all dogs. Table 5 illustrates the mean ± SEM values for hematocrit at the designated times. While the mean values tended to decrease over time, there were no significant differences within or between groups at any time. Note that in both groups, mean values measured at 1 hour were similar to those measured at baseline and postembolization.

The higher mean value for hematocrit in the rt-PA group is explained by the hematocrit of one dog that varied from 55% to 65% over the course of the experiment.

Gas exchange did not deteriorate with embolization or treatment, and values for arterial O\textsubscript{2} tension were similar within and between groups at all times. The lowest recorded value for arterial O\textsubscript{2} tension was 386 mm Hg.

**Discussion**

Our study investigated effects of different dosing regimens of rt-PA on pulmonary thrombolysis and corresponding hemodynamics. We also investigated effects of rt-PA on pulmonary vascular PQ characteristics. The principal finding is that the improvement in pulmonary hemodynamics with rt-PA is explained by a marked decrease in the extrapolated pressure intercept (P\textsubscript{1}) of the pulmonary vascular PQ relation. That is, 1 mg/kg of rt-PA infused over 15 minutes totally abolished the marked increase in P\textsubscript{1} that occurred with embolization. While P\textsubscript{1} also significantly decreased with rt-PA, the change was less than with rt-PA\textsubscript{15}. Furthermore, correlating with the greater improvement in pulmonary hemodynamics with rt-PA, extent of clot lysis, while not significantly different, tended to be greater. Finally, despite a higher concentration of rt-PA during infusion with rt-PA\textsubscript{15}, corresponding rate of thrombolysis was not increased when compared with rt-PA\textsubscript{15}.

Conventionally, pulmonary vascular resistance, calculated as (PAP - LV filling pressure)/CO, is assumed to reflect the flow resistive properties of the pulmonary vasculature, and changes in pulmonary vascular resistance are believed to reflect changes in effective vascular caliber.\textsuperscript{16,17} This calculation uses only a single PQ coordinate to describe the vascular resistance and assumes that the effective pulmonary vascular outflow pressure in Zone III of West is the LV filling pressure.

In the present study, we used the pulmonary artery PQ relation to investigate the effects of pulmonary emboli and rt-PA on pulmonary hemodynamics. The slope of this relation defines the incremental vascular resistance (IR) or the unit pressure change per unit change in flow, and the extrapolated pressure intercept (P\textsubscript{1}) may define the effective vascular outflow pressure.

The effective outflow pressure may be influenced by vascular closing pressure,\textsuperscript{8,11,12} left atrial pressure,\textsuperscript{8,18} alveolar pressure,\textsuperscript{19} or a combination. Several studies have confirmed that under certain conditions, the intercept pressure may exceed LV filling pressure in Zone III\textsuperscript{8,11} and alveolar pressure in Zone II.\textsuperscript{19}

In the present study, the marked increase in PAP with embolization was predominantly due to the increase in P\textsubscript{1}. Identical results, using the same model, are reported in previous studies.\textsuperscript{8-10} One of those studies demonstrated that after embolization, when P\textsubscript{1} was much higher than left atrial pressure (PLa), and despite established Zone III conditions, there was a functional dissociation between PAP and PLa.\textsuperscript{8} Similarly, another group of investigators, using isolated pig lungs, reported a marked increase in P\textsubscript{1} with hypoxia and a dissociation between LAP and PAP.\textsuperscript{11} Finally, a third study, in dogs with normal lungs, reported that when PLa was increased (19 mm Hg) and exceeded baseline P\textsubscript{1}, then P\textsubscript{1} equaled PLa.\textsuperscript{18} These observations support the hypothesis that P\textsubscript{1} reflects the effective vascular outflow pressure. While in West Zone III conditions the mechanism(s) explaining the increase in P\textsubscript{1} has not been determined, recent work in pulmonary

| Table 5. Effect of Embolization and Treatment With rt-PA\textsubscript{15} and rt-PA\textsubscript{15} on Hematocrit |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment      | Baseline        | Postemboli      | 1 hr            | 2 hr            | 3 hr            |
| rt-PA\textsubscript{15} | 36 ± 2          | 36 ± 3          | 35 ± 3          | 31 ± 4          | 30 ± 5          |
| rt-PA\textsubscript{15} | 39 ± 2          | 41 ± 4          | 41 ± 5          | 39 ± 4          | 37 ± 4          |

**Figure 5. Plots of effects of emboli rt-PA\textsubscript{15} and rt-PA\textsubscript{15} on pulmonary artery pressure-flow (PAP)-cardiac output (CO) characteristics in representative dogs.**
embolic hypertension suggests that it may, at least partially, be explained by a disturbed or turbulent flow regimen secondary to localized vasoconstriction and thrombus deposition. In the former study, hydralazine, a nonspecific vasodilator, markedly improved pulmonary hemodynamics, predominantly by decreasing $P_T$. It is also possible that the increase in $P_I$ is partially due to localized pulmonary edema secondary to the acute pulmonary hypertension. While edema may affect $P_I$ in this model, the rapid and marked decrease in intercept pressure with rt-PA suggests its contribution is minor.

The slope of the PQ relation defines the pressure change per unit change in flow or the incremental vascular resistance (IR). The increase in IR in this and previous studies most likely reflects a decrease in effective vascular cross-sectional area, likely due to both embolic obstruction of parallel vascular units and a generalized increase in vascular tone. In contrast to its marked effect on $P_I$, rt-PA had little effect on IR. As illustrated in Table 4, after embolization, IR remained constant over the 3-hour interval. While it is possible IR would have increased if rt-PA had not been given, in previous experiments where only heparin was given after embolization, IR did not increase over approximately 1 hour. Also, a recent study of embolic pulmonary hypertension reported little or no clot lysis and no significant change in PAP or CO over a 3-hour interval in dogs given heparin. These observations argue against a spontaneous, significant increase in IR over the time of this study. They suggest that despite significant clot lysis and a marked decrease in $P_I$ with rt-PA, effective vascular surface area did not significantly increase.

The apparent discrepancy between the decrease in $P_I$ with rt-PA and the failure for IR to improve is likely due to different mechanisms mediating IR and $P_I$ and to the relatively short infusion time of rt-PA. rt-PA has a relatively short circulating plasma half-life—approximately 5 minutes. Therefore, in this study, rt-PA distribution and corresponding intrapulmonary thrombosis should be heavily influenced by the distribution of pulmonary blood flow. That is, the binding of rt-PA to fibrin and subsequent thrombolysis should preferentially occur where vascular obstruction was incomplete. Thrombolysis of clot in partially obstructed vessels would tend to smooth the blood flow profile and thus decrease turbulence and possibly $P_I$. It follows then that rt-PA delivery and thus thrombolysis would be poor in totally obstructed units. Accordingly, with the dosing regimens used, minimal effects on IR could be predicted. This may explain the differential effects of rt-PA on $P_I$ and IR.

While previous work has documented a decrease in PAP with thrombolytic agents, the present study is the first to investigate the effects of a thrombolytic agent on pulmonary PQ characteristics and, thus, on the physiologic mechanism of hemodynamic improvement.

This study also investigated effects of very rapid rt-PA (rt-PA90) infusion versus a more prolonged (rt-PA15) administration on dynamics of thrombolysis. We observed that while both rt-PA90 and rt-PA15 were effective, total thrombolysis and corresponding hemodynamic improvement tended to be greatest with rt-PA15. While previous studies have demonstrated accelerated resolution of thrombi with thrombolytic agents, few have systematically investigated relative efficacy of different dosing regimens. A recent study of canine embolic pulmonary hypertension compared thrombolytic efficacy of 1 mg/kg rt-PA infused over 15 and 90 minutes. While extent of total clot lysis was similar over 3 hours, during drug infusion, rate of thrombolysis and relative decrease in PAP were greatest with rt-PA15. The present study extended these results by demonstrating that despite a threefold increase in infusion rate with rt-PA90, the rate of lysis was similar with rt-PA6 and rt-PA15.

Including our previous work, a total of three different dosing regimens have been tested in this model. The relative initial concentrations of rt-PA achieved during each dosing regimen were calculated by assigning 1 mg/kg over 90 minutes (rt-PA90) a value of 1; therefore, 1 mg/kg over 15 minutes and 5 minutes had values of 6 and 18, respectively. When the initial rate of clot lysis was plotted against the relative concentrations of rt-PA (Figure 6A), the resulting curve suggested that the relation followed Michaelis-Menten kinetics. The linearity of the plot on a double-reciprocal Lineweaver-Burk plot confirms this (Figure 6B). As illustrated in Figure 6B, the Lineweaver-Burk plot allows us to extrapolate a maximum rate of clot lysis of 66%/hr. The close resemblance of the rt-PA dose-response characteristics to the Michaelis-Menten relation may be a useful guide for optimizing the rt-PA dosing regimen. However, further prospective studies are required to confirm this relation.

Note, with respect to the present study, correlating with the trend to greater clot lysis with rt-PA15, pulmonary hemodynamics improved most. As illustrated in Figure 4, from 30 minutes after onset of therapy to the end of the study, the relative decrease in PAP tended to be greatest with rt-PA15; from 45 minutes to 2 hours, this change was significant.

The greater decrease in PAP with rt-PA15 versus rt-PA6 is explained by the more marked effect of rt-PA15 on $P_I$.

Several recent clinical and basic studies investigated the thrombolytic efficacy of rt-PA in venous thromboembolism. Goldhaber et al documented efficacy of intravenous rt-PA in patients with pulmonary embolism. Subsequently, Come et al described effects of rt-PA in seven of the patients from the above study who had right ventricular dysfunction complicating pulmonary embolism. They documented pulmonary thrombolysis and improved right ventricular function. After diagnosis of thrombus, 50 mg rt-PA was
Infused over 2 hours, and a selective pulmonary angiogram was repeated. If significant thrombus remained, additional rt-PA was infused, as tolerated, at 10 mg/hr for 4 hours. The baseline mean angiographic score was 8.1 and at 2 and 6 hours it had decreased 19% and 65%, respectively. While hemodynamic measurements at 2 hours were not reported, by 6 hours there was marked improvement. PAP decreased from 42 to 26 mm Hg and there was a corresponding improvement in right ventricular dimensions. Values for CO were not reported.

One randomized, controlled clinical trial compared rt-PA (100 mg over 2 hours) and urokinase (initial bolus of 4,400 units/kg followed by 4,400 units/kg/hr) in treatment of pulmonary embolism. At 2 hours, thrombolysis and hemodynamic improvement were greatest with rt-PA. The investigators concluded that at the dose regimens used, rt-PA acts more rapidly and is safer than urokinase. Verstraete et al compared intravenous and intrapulmonary rt-PA in treatment of patients with massive pulmonary embolism. After 50 mg rt-PA over 2 hours, the pulmonary angiographic severity score decreased 15% with intravenous rt-PA and 12% with intrapulmonary rt-PA, respectively. Compared with the present study, at 2 hours the rate of thrombolysis was markedly attenuated in the clinical studies. This difference in thrombolytic rate may be explained by the difference in dosing regimens. Other work supports this hypothesis. In an open-label study of patients with acute myocardial infarction, Mueller et al demonstrated a direct relation between the dose of rt-PA and the rate of coronary revascularization. The reperfusion rate was 42% when 50 mg rt-PA was infused over 30 minutes, and this decreased to 24% when 33 mg was given over the same interval. While the decrease in plasma fibrinogen was greatest with the former regimen, there was no difference between groups in bleeding complications.

Considered together, these studies illustrate the important relation between dosing regimen and dynamics of thrombolysis. Because the majority of deaths complicating pulmonary embolism occur within 1 hour of the onset of symptoms, the therapeutic regimen that induces the most rapid rate of thrombolysis appears preferable.

In summary, current results indicate that the improvement in pulmonary hemodynamics with rt-PA is due to complete resolution of the high intercept pressure that occurred with embolization. Furthermore, these results demonstrate that, at least in the model used, there is an upper limit to the relation between the concentration of rt-PA and the rate of thrombolysis.

As cited above, several recent studies have confirmed the thrombolytic efficacy of rt-PA in venous thromboembolism. Present results, those from our recent canine study, and results from the study of patients with acute coronary thrombosis cited above suggest that when circulatory compromise complicates acute pulmonary emboli, relatively rapid infusion of rt-PA may be the preferred method of administration. Also, because there may be an upper limit to the dose thrombolytic rate relation, our results argue against single bolus administration of rt-PA. However, because these results were obtained in an anesthetized canine preparation with exogenously produced autologous blood clots, we emphasize caution in direct extrapolation to patients.

References


Key Words • recombinant tissue-type plasminogen activator • pulmonary embolism • pulmonary pressure-flow relation • pulmonary hypertension
Recombinant tissue-type plasminogen activator in canine embolic pulmonary hypertension. Effects of bolus versus short-term administration on dynamics of thrombolysis and on pulmonary vascular pressure-flow characteristics.

R M Prewitt, F Shiffman, D Greenberg, R Cook and J Ducas

Circulation. 1989;79:929-938
doi: 10.1161/01.CIR.79.4.929

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/79/4/929