Does Tissue-Type Plasminogen Activator Have Direct Beneficial Effects on the Myocardium Independent of Its Ability to Lyse Intracoronary Thrombi?

Robert A. Kloner, MD, PhD, Kevin Alker, Colin Campbell, PhD, Gerald Figures, BSc, Andrew Eisenhauer, MD, and Sharon Hale, BS

Tissue-type plasminogen activator (t-PA) is a widely used thrombolytic agent for treating acute myocardial infarction. Some previous studies suggest that t-PA benefits the heart independently of lysing coronary artery thrombi. The purpose of this study was to determine whether t-PA directly affects infarct size independently of lysing coronary thrombi, affects the no-reflow phenomenon, and exacerbates intramyocardial hemorrhage. We used a canine model of 2 hours of occlusion of the left anterior descending coronary artery followed by 4 hours of reperfusion. t-PA was administered 30 minutes after occlusion and was continued for 2 hours. Myocardial infarct size as a percentage of the risk zone was similar between saline (28±8%) and t-PA (35±9%) groups in a low-dose study and between saline (46±12%) and t-PA (44±12%) groups in a high-dose study. t-PA did not improve no-reflow. Intramyocardial hemoglobin level within the infarct was similar between saline (16 μg/mg) and high-dose t-PA (12 μg/mg) groups. The extent of hemorrhage assessed by intramyocardial hemoglobin correlated with infarct size. Histologic evaluation revealed that microscopic hemorrhage was confined to zones of contraction band necrosis. Neutrophil infiltration during early reperfusion was prominent. In conclusion, t-PA did not directly benefit the myocardium or no-reflow. Its effects in patients are likely due to its ability to lyse thrombi. t-PA did not cause infiltration of hemorrhage into noninfarcted tissue. Reperfusion accelerates the inflammatory response after myocardial infarction and results in early, intense neutrophil infiltration. (Circulation 1989;79:1125–1136)

The 1980s have become the era of coronary artery reperfusion for the treatment of acute myocardial infarction. Several studies have shown that timely thrombolytic therapy decreases chest pain, decreases mortality, improves left ventricular function, and reduces infarct size. Tissue-type plasminogen activator (t-PA) has emerged as one of the most popular thrombolytic agents in the United States, and it has been more efficacious than streptokinase in lysing coronary thrombi. Although t-PA has less effect on the systemic clotting system, the clinical incidence of bleeding complications with t-PA does not appear to be less than with streptokinase. Despite the wide use of t-PA for treating acute myocardial infarction and despite its well-recognized ability to lyse thrombi in proximal coronary arteries, few data are available concerning the effect of t-PA on the myocardium and microvasculature of the heart. Currently, two studies in the literature suggest that the beneficial effects of t-PA on the heart may occur independent of its ability to lyse proximal epicardial coronary artery thrombi. In one study, a coronary artery was occluded with a clamp (mechanical rather than thrombotic occlusion), and t-PA then was administered during the reperfusion phase (release of the clamp). The extent of necrosis in the t-PA group was dramatically reduced, suggesting that t-PA has a direct beneficial effect on the myocardium independent of its ability to lyse proximal coronary thrombi. The investigators suggested three possible mechanisms: 1) induction of vasodilation of coronary arteries, 2) membrane stabilization resulting in the reduced release of autolytic enzymes, and 3) thrombolysis of microthrombi with reduction of no-reflow. A recently reported study in dogs showed similar results.

From the Division of Cardiology, Wayne State University and Harper Hospital, Detroit, Michigan; and The Heart Institute of The Hospital of The Good Samaritan and the Section of Cardiology, University of Southern California, Los Angeles, California.
Address for reprints: Robert A. Kloner, MD, PhD, The Heart Institute of the Hospital of The Good Samaritan, 616 South Witmer Street, Los Angeles, CA 90017.
Received September 30, 1988; revised January 17, 1989.
Coronary artery reperfusion may also result in hemorrhagic myocardial infarction.\textsuperscript{10,11} Thrombolytic agents potentially could exacerbate this hemorrhage into the infarct and perhaps could even result in hemorrhage into nonnecrotic peri-infarct border zones jeopardizing viable myocytes. The purpose of this study was threefold: 1) to determine whether t-PA reduces infarct size independently of its ability to lyse proximal coronary thrombi in a mechanical model of 2 hours of coronary occlusion and 4 hours of reperfusion in the anesthetized dog, 2) to determine whether t-PA exacerbates intramyocardial hemorrhage during coronary reperfusion, and 3) to determine whether t-PA affects the no-reflow phenomenon, assessed with radioactive microspheres.\textsuperscript{12,13}

Two protocols were used in this study. In the first protocol, we used a low dose of t-PA (lower than doses administered to humans but a dose that has been shown to be thrombolytic in the canine model\textsuperscript{15}); in the second protocol, we used a high dose of t-PA (equivalent to doses administered to patients).

**Methods**

**Protocol 1**

Mongrel dogs of either sex were anesthetized with intravenous sodium pentobarbital (35 mg/kg), intubated, and ventilated with a Harvard respirator (South Natick, Massachusetts). Small amounts of additional sodium pentobarbital were administered throughout the experiment as needed to ensure adequate anesthesia. A cannula was positioned into the left carotid artery for monitoring arterial blood pressure and for withdrawing reference blood samples for regional myocardial blood flow (RMBF) measurements. A second catheter was placed into the left jugular vein for administration of fluids and drugs. A left thoracotomy was performed at the fifth left intercostal space, the pericardium was incised, and the left anterior descending coronary artery was then isolated proximal to the first major diagonal branch. A third cannula was positioned in the left atrium for injection of radioactive microspheres for RMBF measurement. After baseline hemodynamic levels were measured, the left anterior descending coronary artery was occluded with a Schwartz atraumatic vascular clamp (Pilling, Ft. Washington, Pennsylvania). RMBF was determined 25 minutes after occlusion (after occlusion, before treatment) with radioactive microspheres.\textsuperscript{13,14}

Thirty minutes after coronary artery occlusion, the surviving dogs were randomized to receive either commercially available t-PA (Activase; 1,000 IU/kg/min; 0.21 mg/kg; Genentech, San Francisco, California) or saline infused intravenously for a total of 2 hours. This administration protocol was followed to simulate the clinical situation in which t-PA is on hand for a period of time before reperfusion. This dose range of t-PA previously has been shown to be thrombolytic in the canine model.\textsuperscript{12}

At 105 minutes after occlusion, RMBF was determined a second time (after occlusion, after treatment). At 120 minutes after occlusion, the coronary artery was reperfused by abrupt removal of the clamp. At 5.5 hours after occlusion, RMBF was determined a third time (after reperfusion). At 6 hours after occlusion (4 hours after reperfusion), the left anterior descending coronary artery was reoccluded briefly at the same site as the initial occlusion, and Monoastral blue dye (0.25 ml/kg, CIBA-GEIGY, Hawthorne, New York) was injected into the left atrium to determine in vivo area at risk. The anesthetized dogs were killed by intracardiac injection of potassium chloride. The heart was excised, the atra and right ventricle were removed, and the left ventricle was sectioned from apex to base into 4–5-mm transverse sections. The heart sections were weighed, and the nonstained zone (area at risk) was traced onto acetate sheets and photographed. The left ventricular sections were then incubated in triphenyltetrazolium chloride (TTC) at 35°C for 10 minutes to delineate the area of necrosis by methods described previously.\textsuperscript{15–17}

The area of necrosis was traced onto acetate sheets and photographed. Area of necrosis and risk were corrected for weight of the left ventricular slices and

![figure](http://circ.ahajournals.org/content/79/5/1126/F1.large.jpg)
were expressed as a percentage of the left ventricle. Area of necrosis also was expressed as a percentage of the area at risk. Samples of myocardium to determine RMbf were cut from the central ischemic and remote nonischemic areas, divided into subepicardium and subendocardium, weighed, and counted in a gamma well counter. Samples of transmural sections of the central ischemic zone were obtained for light microscopy. Sections were examined for the presence of contraction bands, hemorrhage, and neutrophil infiltration. Photographs of heart slices (gross pathology) were projected, and the extent of gross hemorrhage was graded in a semiquantitative fashion as follows: 0 = no gross hemorrhage, 1+ = 25% of infarcted zone hemorrhagic, 2+ = 50% of infarcted zone hemorrhagic, 3+ = 75% of infarcted zone hemorrhagic, and 4+ = 100% of infarcted zone hemorrhagic. Animals used in this study were maintained in accordance with the guidelines prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHEW Publication No. (NIH) 85-23, revised 1985.

### Protocol 2
Protocol 2 was similar to protocol 1 except that dogs were randomized to receive either saline or a higher dose of t-PA (1.3 mg/kg; 754,000 IU/kg, which approximates the currently recommended dose of 100 mg/70 kg) in humans. The treatment was infused intravenously for 2 hours starting 30 minutes after occlusion. In addition, at the end of the protocol, one heart slice that was not incubated in TTC was obtained for determination of hemoglobin content within the area at risk. Hemoglobin was analyzed according to the method of Horecker.18 The area of necrosis for this slice was obtained by using the opposing block face of the adjacent heart slice as a reference.10

In protocols 1 and 2, any dog developing ventricular fibrillation was excluded from the study. In addition, any dogs with pretreatment endocardial blood flow during coronary occlusion greater than 0.40 ml/min/g (high flow) or with risk zones of less than 10% of the left ventricle were excluded because such risk zones result in little if any necrosis in this model.

### Table 1. Hemodynamic Values for Protocol 1

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion</th>
<th>30 min after occlusion (pretreat)</th>
<th>2 hr after occlusion</th>
<th>4 hr after reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>151±7</td>
<td>134±6</td>
<td>132±6</td>
<td>124±7</td>
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<tr>
<td>t-PA (low dose) (n=8)</td>
<td>138±10</td>
<td>141±9</td>
<td>137±11</td>
<td>137±11</td>
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<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>120±5</td>
<td>111±6</td>
<td>112±8</td>
<td>111±6</td>
</tr>
<tr>
<td>t-PA (low dose) (n=8)</td>
<td>124±4</td>
<td>113±5</td>
<td>110±6</td>
<td>108±5</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>107±5</td>
<td>99±7</td>
<td>98±4</td>
<td>100±6</td>
</tr>
<tr>
<td>t-PA (low dose) (n=8)</td>
<td>108±7</td>
<td>100±7</td>
<td>97±7</td>
<td>92±5</td>
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</table>

Data are mean±SEM.

### Table 2. Regional Myocardial Blood Flow for Protocol 1

<table>
<thead>
<tr>
<th></th>
<th>LAD-Epi</th>
<th>LAD-Endo</th>
<th>Cx-Epi</th>
<th>Cx-Endo</th>
</tr>
</thead>
<tbody>
<tr>
<td>After occlusion, before treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>0.28±0.08</td>
<td>0.12±0.03</td>
<td>1.18±0.16</td>
<td>1.20±0.16</td>
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<tr>
<td>t-PA (low dose) (n=8)</td>
<td>0.24±0.06</td>
<td>0.08±0.04</td>
<td>1.03±0.15</td>
<td>1.22±0.20</td>
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<tr>
<td>After occlusion, after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>0.33±0.08</td>
<td>0.17±0.06</td>
<td>1.05±0.08</td>
<td>1.06±0.06</td>
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<tr>
<td>t-PA (low dose) (n=8)</td>
<td>0.33±0.08</td>
<td>0.11±0.04</td>
<td>0.86±0.13</td>
<td>0.92±0.12</td>
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<tr>
<td>After reperfusion, after treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>0.66±0.08</td>
<td>0.37±0.04</td>
<td>0.85±0.12</td>
<td>0.80±0.10</td>
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<tr>
<td>t-PA (low dose) (n=8)</td>
<td>0.58±0.09</td>
<td>0.37±0.06</td>
<td>0.82±0.13</td>
<td>0.77±0.11</td>
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</table>

Data are ml/min/g and are mean±SEM.

LAD-Epi, epicardial blood flow in the myocardium supplied by the left anterior descending coronary artery; LAD-Endo, endocardial blood flow in the myocardium supplied by the LAD; Cx-Epi, epicardial blood flow in the myocardium supplied by the left circumflex coronary artery; Cx-Endo, endocardial blood flow in the myocardium supplied by the Cx.
Statistical Analysis

In both protocols, analysis of variance was used to assess hemodynamic and regional myocardial blood flow changes over time and between groups. Area of necrosis, area at risk, and area of necrosis as a percentage of the area at risk between groups were analyzed by nonpaired $t$ tests. Data are shown as mean±SEM.

Results

Protocol 1

Forty-one dogs were entered into protocol 1. Twenty-two developed ventricular fibrillation (in most cases, before randomization). One dog had no risk zone; one dog had high pretreatment endocardial blood flow during occlusion, and in one dog, the left anterior descending coronary artery was torn during dissection. Of the 16 remaining dogs, eight were randomized to receive saline and eight to receive low-dose t-PA.

The area at risk was similar in both groups at 18±3% of the left ventricle in the saline and 19±2% of the left ventricle in the t-PA group (Figure 1). Area of necrosis as a percentage of the left ventricle was 6±3% in the control group and 7±2% in the t-PA group. Area of necrosis when expressed as a percentage of the area at risk was 28±8% in the saline group and 35±9% in the t-PA group ($p=NS$). When area of necrosis was expressed as a percentage of area at risk and compared with pretreatment epicardial collateral flow within the risk zone (30 minutes after occlusion), there was a negative correlation but no difference between treatment groups (Figure 1B). Therefore, there was no evidence that t-PA reduced infarct size beyond that related to coronary reperfusion.

Hemodynamic data are shown in Table 1. No differences occurred in heart rate and systolic and diastolic blood pressure between the two groups at anytime throughout the protocol.

RMBF data were available in seven of eight dogs receiving saline and in eight of eight dogs receiving t-PA. As expected, RMBF analysis showed that RMBF was depressed in the area at risk during coronary occlusion (Table 2). Collateral blood flow was lower in the subendocardium and higher in the subepicardium. t-PA had no effect on RMBF during coronary occlusion. RMBF levels after reperfusion improved but did not return to normal. RMBF in the endocardium of the area at risk was depressed at 0.37 ml/min/g tissue in saline and t-PA groups, whereas flow in the nonischemic zone averaged 0.77–0.80 ml/min/g. This continued reduction of flow was probably due to the no-reflow phenomenon.

Photographs that were adequate for determining hemorrhagic score were available in seven of eight dogs receiving saline and low-dose t-PA. The extent of gross hemorrhage tended to be larger in the t-PA group (score=1.1±0.4) compared with the saline group (0.6±0.3) although this was not statistically significant. The hemorrhage did not extend beyond the zone of infarction (Figure 2).

Histologic evaluation ($n=16$) of the area at risk revealed the presence of contraction bands typical of irreversibly injured myocytes that have been subject to reperfusion. The extent of microscopic hemorrhage was variable but tended to be more prominent in the t-PA group. Nevertheless, we did not observe zones of microscopic hemorrhage outside of the zone of contraction band necrosis. Hemorrhage was confined to within the area of contraction band necrosis in both groups. Neutrophil infiltration was prominent, especially in the t-PA group. Neutrophils were present both within and peripheral to zones of contraction band necrosis (Figure 3).
FIGURE 3. Panel A: Histologic section from control dog subjected to 2 hours of coronary occlusion and 4 hours of reperfusion. Contraction bands (arrows) are prominent on right-hand side of photomicrograph. In the upper-middle portion is a zone of prominent neutrophil infiltration (arrowhead). There are some interstitial red blood cells in the same region. Panel B: Histologic section from a dog receiving low-dose t-PA. There is extensive necrosis with contraction bands. Neutrophil infiltration is prominent.

Protocol 2

Forty dogs were entered into protocol 2. Twenty-four dogs developed ventricular fibrillation (in most cases, before randomization), three had risk zones less than 10% of the left ventricle, and one dog had high pretreatment endocardial flow during occlu-
sion. Six dogs were successfully randomized to receive high-dose t-PA and six to saline.

The area at risk was similar in both groups at 17±2% of the left ventricle in the control group and 21±2% of the left ventricle in the t-PA group. The extent of necrosis was similar in control and t-PA groups whether expressed as a percentage of the left ventricle (8±2% vs. 10±3%) or as a percentage of area at risk (46±12% vs. 44±12% in control vs. t-PA groups, respectively, p=NS) (Figure 4). Figure 4B shows the relation of area of necrosis to area at risk as a function of ischemic epicardial blood flow within the risk zone. Thus, high-dose t-PA also did not reduce infarct size more than did reperfusion alone.

Histologic analysis (n=12) revealed hemorrhage in both groups (Figure 6). No difference was found in the extent of hemorrhage between groups, and again, hemorrhage tended to be confined to zones of contraction band necrosis and did not extend beyond these areas. Neutrophil infiltration was extensive in both groups (Figure 6). Neutrophils often appeared to be filling vessels and were in the interstitium as well. No noticeable difference was found in the extent of neutrophil infiltration between groups.

Myocardial hemoglobin concentration in nonischemic tissue was 6.5±0.5 μg/mg in the control and 7.0±1.0 μg/mg in the high-dose t-PA group. Within the risk zone, hemoglobin levels were increased in both groups: 16.3±4.5 μg/mg in dogs receiving saline (6 dogs) and 11.6±2.6 μg/mg in dogs receiving t-PA (6 dogs). Hemoglobin levels between control and t-PA groups did not differ. Neither the gross hemorrhagic score, the microscopic, nor the hemoglobin analysis suggested that t-PA exacerbated reperfusion-induced hemorrhage.

A significant correlation was found between the degree of hemorrhage assessed by intramyocardial hemorrhage and area of necrosis (AN), expressed as a percentage of the area at risk (AR) [Hemoglobin = 26.6(AN/AR)+2.0; r=0.85; Figure 7A]. Also, a moderate negative correlation was found between extent of hemorrhage and subepicardial coronary blood flow during occlusion (r=−0.61; Figure 7B), and a negative correlation was found between extent of hemorrhage and subendocardial blood flow after 4 hours of reperfusion (r=−0.66). The degree of hemorrhage did not significantly correlate with heart rate, systolic blood pressure, rate-pressure product, or area at risk.

**Discussion**

t-PA has become a widely used agent for the treatment of acute myocardial infarction. There is no question that it is efficacious in lysing proximal epicardial coronary thrombi and restoring vessel patency. Coronary reperfusion, when instituted within a few hours of coronary occlusion, reduces infarct size. Early investigators had assumed that any beneficial effect that t-PA had on the myocardium was through its ability to lyse proximal thrombi and to initiate reperfusion. However, a study by Darius et al suggested that t-PA may reduce the extent of myocardial necrosis by a mechanism that is independent of its ability to lyse proximal thrombi. They used a melanoma cell-derived tissue-type plasminogen activator in a cat model of 2 hours of coronary occlusion followed by
4 hours of reperfusion. t-PA was infused at a dosage of 500 IU/kg/min for the first 30 minutes of reperfusion. These investigators also showed that the mass of necrosis was lower in the t-PA group (30% of the area at risk) compared with that in the placebo group (47% of the area at risk) \((p<0.02)\). These investigators postulated that t-PA had a direct protective effect on the myocyte or that t-PA dissolved microthrombi. Although coronary vasodilation was another proposed mechanism, these investigators reported that in isolated, perfused coronary arteries of cats, plasminogen activator did not show any vasodilator properties. Berger et al\(^9\) also investigated this “direct salvaging” activity of t-PA in a canine model. Dogs were subjected to 90 minutes of mechanical coronary occlusion followed by 24 hours of reperfusion that was induced by the gradual release of a snare. Four treatment groups were studied: control, t-PA (750,000 IU/kg), the oxygen radical scavenging agent superoxide dismutase, and combination of t-PA and superoxide dismutase. The treatment was started 15 minutes before reperfusion and continued for 1 hour. Myocardial infarct size expressed as a percentage of the risk zone was 35% in the control group and was reduced to 14% in the t-PA group. Superoxide dismutase alone also reduced infarct size in this study (13%), but a striking finding was that the combination of t-PA and superoxide dismutase reduced infarct size to only 1.7% of the risk zone. Those investigators concluded that t-PA could directly salvage ischemic myocardium independently of its thrombolytic activity.

Jaffe\(^21\) pointed out in an editorial, that if t-PA reduces necrosis by “mechanisms other than lysis of coronary thrombi,” the “implications are profound.” Such findings would suggest that some of the beneficial effects seen with t-PA such as improved left ventricular function\(^22\) and relatively low mortality\(^4\) in patients receiving this drug may have nothing to do with lysis of proximal intracoronary thrombi but may be related to direct myocardial cellular mechanisms.

In the present study, we did not show any evidence of a direct protective effect of t-PA on the

### Table 3. Hemodynamic Values for Protocol 2

<table>
<thead>
<tr>
<th></th>
<th>Preocclusion</th>
<th>30 min after occlusion (pretreat)</th>
<th>2 hr after occlusion</th>
<th>4 hr after reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ((n=6))</td>
<td>141±4</td>
<td>137±5</td>
<td>130±8</td>
<td>128±13</td>
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<tr>
<td>t-PA (high dose) ((n=5))</td>
<td>156±10</td>
<td>140±7</td>
<td>133±7</td>
<td>128±11</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
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<td></td>
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<tr>
<td>Control ((n=6))</td>
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<td>111±12</td>
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<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
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<td>116±12</td>
<td>110±11</td>
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<td>84±10</td>
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Data are mean±SEM.

### Table 4. Regional Myocardial Blood Flow for Protocol 2

<table>
<thead>
<tr>
<th></th>
<th>LAD-Epi</th>
<th>LAD-Endo</th>
<th>Cx-Epi</th>
<th>Cx-ENDO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>After occlusion, before treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ((n=5))</td>
<td>0.11±0.05</td>
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<td>1.20±0.16</td>
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<td><strong>After occlusion, after treatment</strong></td>
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<tr>
<td>Control ((n=6))</td>
<td>0.21±0.09</td>
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<td><strong>After reperfusion, after treatment</strong></td>
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<td>0.76±0.11</td>
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</table>

Data are ml/min/g and are mean±SEM.

LAD-Epi, epicardial blood flow in the myocardium supplied by the left anterior descending coronary artery; LAD-Endo, endocardial blood flow in the myocardium supplied by the LAD; Cx-Epi, epicardial blood flow in the myocardium supplied by the left circumflex coronary artery; Cx-Endo, endocardial blood flow in the myocardium supplied by the Cx.
myocardium in the anesthetized canine model of mechanical coronary occlusion followed by reperfusion. We used two doses of t-PA. In one protocol, a low dose of t-PA was used that previously had been shown to be thrombolytic in a canine model.14 We also used a higher dose of t-PA that is equivalent to that administered to patients (equivalent to 100 mg given to a 70 kg person). We observed no effect with either dosage regimens on the extent of necrosis whether expressed as a percentage of the left ventricle or normalized to the size of the area at risk.

Thus, our results suggest that any beneficial effects of t-PA result from its ability to lyse proximal thrombi rather than to some direct protective effect on the myocardial cells. Our results also do not support the hypothesis that t-PA's mechanism of action is by dissolution of microthrombi that results in improved perfusion of the coronary microvasculature. Reperfusion myocardial blood flows were depressed, especially in the subendocardium. This reduction in flow was most likely related to the no-reflow phenomenon.25 Neither low- nor high-dose t-PA improved reperfusion regional blood flows compared with control groups, which suggests that t-PA had no effect on microvascular perfusion or no-reflow phenomenon. We observed similar results in a previous study with streptokinase.10 Streptokinase, in a mechanical model of coronary occlusion and reperfusion, failed to reduce myocardial infarct size. Therefore, the benefits to myocardium of both streptokinase and t-PA are likely due to epicardial coronary artery recanalization rather than to a direct effect on myocardial cells or the microvasculature.

Some technical differences do exist between the negative findings of the present study and the positive findings of the two previous studies that may explain the different outcomes. The study by Darius et al8 was in a cat model; therefore, species differences may account for the different results. Also, they used a melanoma cell–derived tissue-type plasminogen activator that may have actions different from the single-stranded, commercially available (Genentech) t-PA that was used in the present study. Also, a recent study from the same laboratory24 failed to show a benefit of t-PA on infarct size. However, a combination of t-PA and a thromboxane synthetase inhibitor potentiated myocardial salvage in this study. The reason for a failure of t-PA alone to reduce infarct size in their second study is not clear. Collateral blood flow was not measured in the study by Darius et al.8 Conceivably, a higher baseline collateral blood flow in several cats in the t-PA group may have contributed to an apparent reduction in infarct size. Berger et al6 induced reperfusion by using a technique different from the one used in the present study. They gradually released the snare to prevent hyperemia, whereas we simply removed the atraumatic vascular clamp around the left anterior descending coronary artery. It is unknown whether differences in the t-PA preparation (Burroughs Wellcome) used in the study by Berger et al6 and the t-PA preparation (Genentech) used in our study could explain the different outcomes.

Our study, that of Darius et al8 and that of Berger et al6 used a mechanical means of inducing coronary artery occlusion. The effect of t-PA in a thrombotic occlusion model may be quite different. In addition to lysing a proximal epicardial coronary thrombus and allowing blood reperfusion, t-PA, theoretically, could lyse any microvascular emboli originating from the original thrombus. Because an anesthe-
tized animal preparation was used, we cannot rule out the possibility that drug-to-drug interaction somehow inhibited a positive effect. However, anesthesia alone probably could not explain the negative results of our study because anesthetized preparations were also used in the two previous studies that had positive results with t-PA.6,9

Hemorrhage into the myocardium is a complication of coronary reperfusion. This phenomenon has been documented by gross pathologic study, microscopy, and measurement of myocardial hemoglobin levels.10,11,25 Probably, the vascular endothelium becomes damaged during ischemia. During reperfusion, the vascular endothelium can no longer contain the influx of blood, and intramyocardial hemorrhage occurs. Whether hemorrhage after reperfusion is deleterious remains controversial. If the hemorrhage within the infarct were to infiltrate the viable myocardium, then the pressure exerted by this hemorrhage in the interstitium could jeopardize blood supply to the still viable myocytes. In the experimental canine model of coronary occlusion followed by reperfusion, including the present study, we have not observed hemorrhage infiltrating areas beyond the myocardium already damaged. Although hemorrhage theoretically could impede the healing process of reperfused myocardium, we have not observed long-term deleterious consequences of scar formation in reperfused infarcts.11 Although there was a nonsignificant trend for increased hemorrhage in the low-dose t-PA group compared with the control group, this was not observed in the high-dose t-PA group. In the high-dose study (which used a dose approximately equivalent to the dose currently used in humans), hemorrhage was extensive but equally severe in control and t-PA groups. Similar results previously were reported by our laboratory with streptokinase.10

A striking histologic feature in the reperfused myocardium of control and t-PA groups in both protocols was the prominent influx of neutrophils. This finding suggests that reperfusion of infarcts alters the development of the inflammatory response. Although the classic studies of pathology of infarcts show peak neutrophil infiltration at approximately 48 hours after coronary occlusion,26 reperfusion appears to speed this initial inflammatory response. Thus, after 2 hours of coronary occlusion and only 4 hours of reperfusion, neutrophil infiltration was prominent. Large sheets of neutrophils were in the interstitium and appeared to fill some capillaries. Some investigators have postulated that these neu-
trophils may contribute to so-called "reperfusion injury" by acting as a source of oxygen radicals or contribute to the no-reflow phenomenon. The early and intense influx of neutrophils that we observed microscopically confirms studies by Engler et al., who used indium-labeled neutrophils in a model of occlusion and reperfusion.

In summary, the present study did not reveal any direct beneficial effect of t-PA on the myocardium. The beneficial effects of the agent probably are due to its ability to lyse proximal epicardial coronary thrombi and not related to some direct myocardial protective effect or preservation of microvascular flow by lysing microthrombi. Hemorrhage is a feature of reperfused myocardial infarcts; however, even when reperfusion occurred during t-PA infusion in this study, hemorrhage did not appear to infiltrate areas of myocardium that were still viable. Finally, coronary reperfusion appears to accelerate neutrophil infiltration, which is very prominent after only a relatively brief period of reperfusion.

Acknowledgment

We are grateful to Genentech for supplying the t-PA used in this protocol.

References


FIGURE 7. Panel A: Plot of correlation between myocardial hemoglobin concentration and area of necrosis (AN) expressed as a percentage of the area at risk (AR). ○, both groups. Panel B: Correlation between myocardial hemoglobin concentration and subepicardial myocardial blood flow during ischemia; LAD, left anterior descending coronary artery; □, control; ●, high-dose t-PA.


**KEY WORDS** • coronary reperfusion • myocardial infarct size • myocardial hemorrhage • neutrophils
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R A Kloner, K Alker, C Campbell, G Figures, A Eisenhauer and S Hale

Circulation. 1989;79:1125-1136
doi: 10.1161/01.CIR.79.5.1125

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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:
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