Differential Sensitivity of Erythrocyte-Rich and Platelet-Rich Arterial Thrombi to Lysis With Recombinant Tissue-Type Plasminogen Activator

A Possible Explanation for Resistance to Coronary Thrombolysis

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Acute myocardial infarction is triggered by coronary artery occlusion that may be recanalized by thrombolytic therapy with a success rate of up to 75% only. The resistance of coronary artery occlusion to thrombolysis may either be due to obstruction of the lumen by a nonthrombotic mechanism or by intrinsic resistance of thrombus to dissolution. Coronary arterial thrombi are composed of platelet-rich and erythrocyte-rich material in variable proportions. To evaluate the relative sensitivity of these thrombus components to thrombolysis, we have used two femoral arterial thrombosis models in the rabbit, consisting of erythrocyte-rich clot produced by injecting whole blood and thrombin in an isolated segment and of platelet-rich thrombus spontaneously formed on an everted (inside out) femoral arterial segment. Intravenous infusion of recombinant tissue-type plasminogen activator (rt-PA) at a rate of 30 μg/kg/min consistently reperfused arteries occluded with erythrocyte-rich clot (six of six animals compared with zero of six placebo-treated animals, p=0.002), whereas infusion of 30 or 100 μg/kg/min was significantly less efficient for reperfusion of everted segments occluded with platelet-rich material (only four of 12 animals, p=0.01). Intra-arterial infusion proximal to the occlusion, at a rate of 20 μg/kg/min reperfused six of seven rabbits with erythrocyte-rich clots but only one of seven rabbits with occluded everted segments (p=0.03). A dose of 100 μg/kg/min was necessary to reperfuse platelet-rich occlusions in five of six rabbits. We conclude that platelet-rich arterial thrombus is much more resistant to thrombolysis with rt-PA than erythrocyte-rich clot. This differential sensitivity to lysis may explain the failure of thrombolytic therapy in a significant percentage of patients with acute myocardial infarction who may have a predominantly platelet-rich occlusion. The rabbit femoral arterial evasion graft model may represent a useful tool for developing strategies directed at the dissolution of platelet-rich thrombus. (Circulation 1989;79:920–928)

Thrombolytic therapy in patients with acute myocardial infarction may reperfuse occluded coronary arteries, reduce infarct size, preserve left ventricular function, and decrease mortality.1 Thrombolytic therapy with intravenous recombinant tissue-type plasminogen activator (rt-PA) will only reperfuse approximately 75% of occluded arteries,2–6 a ceiling that cannot be increased by combined infusion of therapeutic doses of rt-PA and urokinase,7 or by intracoronary administration of streptokinase.2 This resistance to thrombolysis of approximately 25% of coronary artery
occclusions may be due to luminal compression by intraplaque hemorrhage or coronary arterial dissection or to intrinsic resistance of the clot to lysis, which occurs as a result of variable clot architecture or composition.

Postmortem microscopic examination of serial sections of coronary thrombus of patients with acute myocardial infarction and sudden death has revealed that thrombus formed at the plaque fissure is very rich in platelets, whereas proximal and distal extensions of the thrombus are composed of erythrocyte-rich material.8 Thus, both platelet-rich and erythrocyte-rich zones are present in coronary thrombus, occasionally in several layers,9 and their relative contribution to thrombus mass and to occlusion of the cross-sectional luminal area may vary.

Preformed whole blood or plasma clots immersed in citrated plasma in vitro are readily lyzed with tissue-type plasminogen activator (t-PA) at concentrations of a few micrograms per milliliter,10 whereas platelet aggregates induced in platelet-rich plasma require at least a tenfold higher concentration of rt-PA for disaggregation.11 This indicated to us that platelet-rich regions of coronary clot may be more resistant to lysis than erythrocyte-rich regions and that resistance of coronary thrombus to lysis may, at least in part, be due to a relative preponderance of platelet-rich material.

Therefore, we investigated the efficacy of rt-PA for arterial reperfusion in two arterial thrombosis models in the rabbit, one consisting of occlusive whole blood clot and the other primarily composed of platelet-rich material.

**Methods**

**Reagents**

rt-PA (Activase) was supplied by Genentech, South San Francisco, California.

**Femoral Artery Eversion Graft Model**

(Platelet-Rich Thrombus Model)

Initially, the model developed by Hergrueter et al12 was used with minor modifications as described below. New Zealand White rabbits weighing from 2.2 to 3.4 kg were anesthetized by intramuscular injection of 2/3 ml ketamine (Ketalar, 100 mg/ml, Parke-Davis, Morris Plains, New Jersey) and 1/3 ml xylazine (Rompun, 100 mg/ml, Mobay, Shawnee, Kansas). A groin incision was made to expose the femoral artery between the inguinal ligament and the distal bifurcation. Small muscular side branches were cauterized with a bipolar cautierizer (Codman and Shurtleff, Randolph, Massachusetts). The epigastric artery was cannulated with a Silastic tubing (i.d., 0.012 in., Dow Corning, Midland, Michigan) for intra-arterial administration of rt-PA or placebo. A 5-mm segment of the right femoral artery was excised between two ligation, stripped of excessive adventitial tissue, everted outside-out, and inserted in the left femoral artery under a surgical microscope (Wild M651, Heerbrugg, Switzerland) by end to end anastomosis with 12 interrupted sutures with 10-0 nylon (Sharpoint, Reading, Pennsylvania). The microvascular clamp that clamped off the proximal and distal ends of the transsected artery was released, the vessel was observed for 10 minutes, and the patency assessed by distal milking at 5-minute intervals as follows. The femoral artery was occluded distally to the eversion graft by external compression with blunt forceps. A segment of approximately 1 cm was emptied of blood by compression with a second forceps that was moved distally. Then, the proximal forceps was released to observe whether or not the empty segment was filling with blood through the everted arterial segment. The contralateral femoral vein was cannulated for baseline blood sampling and for intravenous infusion of rt-PA or placebo with a constant rate infusion pump (Syringe infusion pump 22, Harvard Apparatus, South Natick, Massachusetts).

After an initial series of experiments in 14 rabbits, the model was improved in the following ways (Figure 1). Anesthesia was performed with intravenous injection of sodium pentobarbital (35 mg/kg followed by 10 mg at 30–60-minute intervals) through a marginal ear vein or femoral vein. The right brachial artery was cannulated with Intracath 23 gauge (Deseret Medical, Becton Dickinson, Sandy, Vermont) for blood pressure monitoring and blood sampling 50 minutes after the start of the infusion. Blood flow in the left femoral artery containing the everted graft was continuously monitored with an ultrasonic flowmeter (T101 Ultrasonic blood flowmeter, Transsonic Systems, Ithaca, New York). When the flow had decreased to less than 0.5 ml/min, the vessel was considered occluded, and heparin was given through the cannulated femoral vein (bolus of 200 units/kg followed by 70 units/kg at hourly intervals). Occlusion occurred within 15 minutes in approximately 70% of the animals. In the rabbits with persistent patency at 15 minutes, the everted segment was damaged by external compression with blunt forceps for 1–2 seconds, once or twice at 5-minute intervals, whereby occlusion was consistently obtained.

Ten minutes after occlusion, rt-PA (20, 30, or 100 μg/kg/min) or placebo at a total volume of 8 ml/kg body wt was infused through the cannulated femoral vein (intravenous route) or superficial epigastric artery (intra-arterial route) for 60 minutes with the use of a Harvard infusion pump. Reperfusion was defined as a blood flow of more than 25% of the baseline flow. If the everted arterial segment was open at the end of the 1-hour infusion period, flow was monitored for an additional hour. At the end of the experiment, the everted femoral arterial segment and the adjacent regions were opened longitudinally for visual inspection of thrombotic material. The segment was then fixed in either formaldehyde or glutaraldehyde for pathologic examination.
Whole Blood Clot Model (Erythrocyte-Rich Thrombus Model)

New Zealand White rabbits were anesthetized in initial experiments with ketamine and xylazine but subsequently with pentobarbital as described above. The left femoral artery and the right femoral vein were exposed. The right femoral vein and the left deep femoral artery were cannulated with Silastic tubing (i.d., 0.020 in.) and the left superficial epigastric artery with Intracath (23 gauge) (Figure 2). The right brachial artery was also cannulated with Intracath for blood pressure monitoring and blood sampling at 50 minutes of infusion. A 1-cm segment of the artery was clamped proximally and distally to the superficial epigastric artery, and the isolated segment was emptied through the side-branch catheter.

Bovine thrombin (0.05 ml Thrombina 5,000 units/vial, Armour Pharmaceutical, Kankakee, Illinois) and blood (0.1 ml) taken from the right femoral vein were injected through the left epigastric artery cannula after damaging the intima by repeated exter-
nal compression with a blunt forceps. Ten minutes later, first the proximal and then the distal clamp was released. Immediately after confirming total occlusion, heparin was administered as described above. Ten minutes later, infusion of rt-PA or placebo was started through the right femoral vein or the cannulated left deep femoral artery. Blood flow was monitored with the ultrasonic flowmeter throughout the experiment. At the end of the experiment, the segment was visually examined and fixed for pathologic examination.

**Evaluation of Platelet Aggregation**

Six rabbits were anesthetized with sodium pentobarbital and 10 ml blood was collected through the femoral vein into plastic tubes containing 100 μl of 40% trisodium citrate for platelet aggregation studies. rt-PA was then infused at a rate of 20 μg/kg/min for 30 minutes through the cannulated femoral vein. After 25–30 minutes of the infusion, blood was collected for platelet aggregation through the cannulated right brachial artery. At the end of the first infusion, the infusion rate was increased to 80 μg/kg/min for 30 minutes, and blood was again collected toward the end of the second infusion.

Platelet-rich plasma was obtained by centrifugation at 800g for 3 minutes in an HN-SII Bench-Top centrifuge (IEC Model 2355, Damon/IEC, Needham, Massachusetts) and platelet aggregation was monitored within 60 minutes of collecting the blood in a platelet aggregometer (Chrono-Log Corp, Havertown, Pennsylvania) after adding 2 or 18 μM ADP. Disaggregation of ADP-induced platelet aggregates was measured after addition of rt-PA to a final concentration of 5 or 100 μg/ml plasma, at the time point where platelet aggregation was maximal.

**Hematologic Assays**

Blood samples for hematologic analysis were obtained through the femoral vein at baseline before injecting heparin and through the brachial artery 50 minutes after the starting infusion. These samples were used for determining hematocrit level, partial thromboplastin time, fibrinogen assay, and rt-PA antigen level.

**Pathologic Examination**

At the end of the experiment, the rabbits were killed with an overdose of pentobarbital. Segments of the thrombosed or reperfused femoral artery were removed and fixed overnight in 5% formaldehyde. The segments were sectioned longitudinally, stained with hematoxylin and eosin or with PTAH (phosphotungstic acid hematoxylin) and evaluated microscopically. Some segments of reperfused arteries were isolated intact and subjected to perfusion fixation with glutaraldehyde and scanning electron microscopy. The artery segment was prepared as previously described.

**Statistical Analysis**

The significance of differences between groups was determined with Fisher's exact test or with Student's t test. Data are recorded as mean ± SD.

**Results**

Table 1 shows the results of femoral arterial perfusion status at the end of the 1-hour infusion and at the end of the experiment. In the first series of experiments, with ketamine and xylazine anesthesia and assessment of perfusion status with the distal milking technique, intra-arterial infusion of rt-PA at a rate of 20 μg/kg/min for 60 minutes caused reperfusion in six of seven rabbits with erythrocyte-rich whole blood clots and only one of seven with platelet-rich everted graft occlusions (p=0.03). Reocclusion during the 1-hour observation period after the end of the infusion was observed in one of three reperfused rabbits with whole blood clot occlusions, despite intensive heparin anticoagulation that prolonged the partial thromboplastin time to more than 100 seconds. However, the

**Table 1. Frequency of Femoral Artery Reperfusion and Reocclusion in the Whole Blood Clot and Eversion Graft Thrombosis Models**

<table>
<thead>
<tr>
<th>Anesthesia</th>
<th>Agent</th>
<th>Route</th>
<th>Dosage (μg/kg/min)</th>
<th>Whole blood clot</th>
<th>Eversion graft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reperfusion</td>
<td>Reocclusion</td>
<td>Reperfusion</td>
</tr>
<tr>
<td>ketamine</td>
<td>rt-PA</td>
<td>i.a.</td>
<td>20</td>
<td>6/7</td>
<td>1/3</td>
</tr>
<tr>
<td>and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylazine</td>
<td></td>
<td>i.v.</td>
<td>0/6</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>pentobarbital</td>
<td>placebo</td>
<td>i.a.</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.v.</td>
<td>30</td>
<td>6/6†</td>
<td>0/6</td>
</tr>
<tr>
<td>rt-PA</td>
<td></td>
<td></td>
<td>100</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td>i.a.</td>
<td>30</td>
<td>...</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>...</td>
<td>5/6§</td>
</tr>
</tbody>
</table>

Data represent number of animals reperfused per total number studied in the group.

rt-PA, recombinant tissue-type plasminogen activator; i.v., intravenous; i.a., intra-arterial.

*p=0.03 vs. i.a. rt-PA in the whole blood clot model (6/7); †p=0.002 vs. placebo (0/6); ‡p=0.01 vs. i.a. rt-PA in the whole blood clot model (6/6), and p=0.6 vs. placebo in the eversion graft model (1/6); §p=0.08 vs. placebo in the eversion graft model (1/6).

†The fifth animal was killed at the end of the infusion to investigate the graft by scanning electronmicroscopy.
combination of ketamine and xylazine reduced systolic arterial blood pressure from an average of 110 to 65 mm Hg in three rabbits. Therefore, pentobarbital anesthesia was used in all subsequent experiments, and blood flow and arterial pressure were measured continuously with the improved protocol outlined under methods.

These experiments with placebo or rt-PA at 30 or 100 μg/kg/min were performed in a randomized fashion for each model and route of administration, with the investigator who performed the animal experiments unaware of the agent and dose used. The occlusions in both the whole blood clot (erythrocyte-rich) and in the eversion graft (platelet-rich) thrombosis models were stable as shown by the results obtained with placebo infusion. Intravenous infusion of 30 μg/kg/min rt-PA caused reperfusion in six animals with erythrocyte-rich clots within 16±13 minutes, and no reocclusion occurred during the follow-up period. Reperfusion restored blood flow from a baseline value of 8.6±2.5 ml/min to a postreperfusion value of 7.2±2.9 ml/min. This reperfusion frequency is significantly different from that in the placebo group, in which none of six animals were reperfused spontaneously (p=0.002). Intravenous infusions of rt-PA in the platelet-rich clots at a rate of 30 μg/kg/min reperfused only two of six animals within 33 and 50 minutes, whereas an increased dosage of 100 μg/kg/min also only reperfused two of six animals within 14 and 50 minutes. The difference in the reperfusion rate after intravenous rt-PA (six of six animals with erythrocyte-rich clots compared with four of 12 rabbits with platelet-rich thrombus) is significant (p=0.01). Intra-arterial administration of rt-PA at a rate of 20 μg/kg/min caused reperfusion in six of seven rabbits with erythrocyte-rich clots but in only one of seven rabbits with platelet-rich thrombus (p=0.03). Only with an increased intra-arterial infusion rate of 100 μg/kg/min was reperfusion obtained in five of six rabbits (p=0.08 compared with placebo group). In the eveted graft model, reocclusion occurred almost consistently during the 1-hour follow-up period.

Table 2 shows results of hemostasis and hemodynamic variables in the groups anesthetized with sodium pentobarbital. None of the infusion schemes induced a marked decrease of blood pressure or of the hematocrit level during the experimental observation period. Placebo infusions did not induce changes in fibrinogen levels. Intravenous infusion of rt-PA at 30 μg/kg/min at 50 minutes resulted in a plasma level of 2 to 3 μg/ml, which was not associated with extensive fibrinogen breakdown (residual fibrinogen level 80–90%). Intravenous infusion of rt-PA at 100 μg/kg/min produced a much higher plasma rt-PA level, that was associated with extensive systemic fibrinogen degradation, resulting in a residual fibrinogen level of 21±11% of baseline at 50

### Table 2. Hematologic and Hemodynamic Variables

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Route</th>
<th>Dosage (μg/kg/min)</th>
<th>t-PA Ag at 50 min (μg/ml)</th>
<th>Fibrinogen level Before (g/l)</th>
<th>50 min (g/l)</th>
<th>Residual (%)</th>
<th>Hematocrit level Before (%)</th>
<th>50 min (%)</th>
<th>Systolic BP Before (mm Hg)</th>
<th>50 min (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood clot model</td>
<td>Placebo</td>
<td>ND</td>
<td>1.8±0.3</td>
<td>1.8±0.3</td>
<td>103±6</td>
<td>39±2</td>
<td>39±1</td>
<td>101±5</td>
<td>101±8</td>
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<tr>
<td></td>
<td>rt-PA</td>
<td>i.v.</td>
<td>30</td>
<td>1.9±0.3</td>
<td>1.5±0.1</td>
<td>81±11</td>
<td>38±1</td>
<td>38±2</td>
<td>101±5</td>
<td>101±8</td>
</tr>
<tr>
<td>Everted graft model</td>
<td>Placebo</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>39±1</td>
<td>38±2</td>
<td>111±9</td>
<td>107±13</td>
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<tr>
<td></td>
<td>rt-PA</td>
<td>i.v.</td>
<td>30</td>
<td>2.1±0.3</td>
<td>1.8±0.2</td>
<td>1.6±0.2</td>
<td>90±7</td>
<td>38±2</td>
<td>37±2</td>
<td>103±6</td>
</tr>
<tr>
<td></td>
<td>rt-PA</td>
<td>i.v.</td>
<td>100</td>
<td>22±5.1</td>
<td>1.7±0.1</td>
<td>0.4±0.2</td>
<td>21±11</td>
<td>38±4</td>
<td>36±4</td>
<td>107±7</td>
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<tr>
<td></td>
<td>rt-PA</td>
<td>i.a.</td>
<td>30</td>
<td>0.7±0.8</td>
<td>1.8±0.3</td>
<td>1.5±0.3</td>
<td>85±14</td>
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<td>36±3</td>
<td>111±8</td>
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<tr>
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<td>rt-PA</td>
<td>i.a.</td>
<td>100</td>
<td>1.8±1.9</td>
<td>1.7±0.1</td>
<td>1.2±0.2</td>
<td>71±10</td>
<td>39±2</td>
<td>34±2</td>
<td>114±7</td>
</tr>
</tbody>
</table>

Data are mean±SD.
BP, blood pressure; rt-PA, recombinant tissue-type plasminogen activator; ND, not determined.

### Table 3. Detailed Results Obtained With the Eversion Graft Model

<table>
<thead>
<tr>
<th>Group</th>
<th>Route</th>
<th>Dosage (μg/kg/min)</th>
<th>Baseline flow (ml/min)</th>
<th>Everted segment length (min)</th>
<th>Time to occlusion (min)</th>
<th>n</th>
<th>Reperfusion Time (min)</th>
<th>Flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>i.a.</td>
<td>...</td>
<td>12±1.8</td>
<td>6.2±0.4</td>
<td>11±6.6</td>
<td>1</td>
<td>28</td>
<td>5</td>
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<tr>
<td>rt-PA</td>
<td>i.v.</td>
<td>30</td>
<td>16±3.2</td>
<td>5.5±0.5</td>
<td>11±12</td>
<td>2</td>
<td>33,50</td>
<td>6, 9</td>
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<tr>
<td></td>
<td>100</td>
<td>15±4.4</td>
<td>5.1±0.5</td>
<td>11±10</td>
<td>2</td>
<td>14,50</td>
<td>4, 8</td>
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</tr>
<tr>
<td>rt-PA</td>
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<td>30</td>
<td>13±4.2</td>
<td>6.4±0.4</td>
<td>6±5</td>
<td>3</td>
<td>24±29</td>
<td>8±7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>11±1.4</td>
<td>6.2±0.3</td>
<td>12±12</td>
<td>5</td>
<td>9±7</td>
<td>8±3</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SD.
rt-PA, recombinant tissue-type plasminogen activator.
Table 4. ADP-Induced Platelet Aggregation and Disaggregation After In Vitro Addition or In Vivo Infusion of rt-PA

<table>
<thead>
<tr>
<th>ADP 18 μM</th>
<th>n</th>
<th>Maximal changes (ΔT)</th>
<th>Aggregation slope (ΔT/min)</th>
<th>Disaggregation slope (ΔT/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo rt-PA infusion</td>
<td>Baseline</td>
<td>6</td>
<td>50±7.8</td>
<td>100±17</td>
</tr>
<tr>
<td></td>
<td>20 μg/kg/min</td>
<td>5</td>
<td>48±9.6</td>
<td>100±16</td>
</tr>
<tr>
<td></td>
<td>80 μg/kg/min</td>
<td>5</td>
<td>48±8.7</td>
<td>110±22</td>
</tr>
<tr>
<td>In vitro rt-PA addition</td>
<td>5 μg/ml</td>
<td>6</td>
<td>50±4.4</td>
<td>110±16</td>
</tr>
<tr>
<td></td>
<td>100 μg/ml</td>
<td>6</td>
<td>44±4.9</td>
<td>110±22</td>
</tr>
<tr>
<td>ADP 2 μM</td>
<td>In vivo rt-PA infusion</td>
<td>Baseline</td>
<td>6</td>
<td>35±6.8</td>
</tr>
<tr>
<td></td>
<td>20 μg/kg/min</td>
<td>5</td>
<td>28±13</td>
<td>120±62</td>
</tr>
<tr>
<td></td>
<td>80 μg/kg/min</td>
<td>5</td>
<td>29±11</td>
<td>140±30</td>
</tr>
<tr>
<td>In vitro rt-PA addition</td>
<td>5 μg/ml</td>
<td>6</td>
<td>38±6.5</td>
<td>150±34</td>
</tr>
<tr>
<td></td>
<td>100 μg/ml</td>
<td>6</td>
<td>40±4.7</td>
<td>160±30</td>
</tr>
</tbody>
</table>

Data are mean±SD.
rt-PA, recombinant tissue-type plasminogen activator. ΔT, change in transmission.
*p=0.03 vs. baseline; †p=0.01.

minutes. Intra-arterial infusion of rt-PA both at 30 μg/kg/min and at 100 μg/kg/min produced lower systemic rt-PA levels and less extensive systemic fibrinogen breakdown than the comparable intravenous infusions. This is probably due to a significant loss of rt-PA by slow leakage through the proximal anastomosis during the arterial infusion. However, systemic rt-PA levels produced by intra-arterial infusion of rt-PA at 100 μg/kg/min were comparable to those obtained with intravenous infusion of rt-PA at 30 μg/kg/min. Partial thromboplastin times were in excess of 100 seconds in all animals.

Table 3 shows some more detailed results obtained in the arterial eversion graft model. Baseline flow in the femoral artery averaged 11–16 ml/min. The length of the everted segment was between 5.1 and 6.4 mm as determined by micrometry under the surgical microscope. This segment length was significantly shorter than the 10.5 to 10.7 mm segment isolated for production of the whole blood clot. The time to occlusion ranged between groups from 6 to 12 minutes. Reperfusion, when observed, restored blood flow to approximately 50% of the baseline value.

Table 4 shows results of ADP-induced platelet aggregation and the effects of infusion or in vitro addition of rt-PA on platelet disaggregation. When platelet aggregation was induced with 18 μM ADP, extensive rapid aggregation was observed with no change after in vivo infusion of rt-PA and a small but significant disaggregation after in vitro addition of 100 μg/ml of rt-PA (p=0.03 compared with baseline). With 2 μM ADP, platelet aggregation was less extensive, but rapid and some spontaneous disaggregation was observed. In vivo rt-PA infusion did not influence the rate nor extent of platelet aggregation, but disaggregation was significantly augmented after in vitro addition of rt-PA to a final concentration of 100 μg/ml (p=0.01). Figure 3 illustrates platelet aggregation curves obtained in one representative experiment.

Pathologic Examination

Figure 4 represents photomicrographs of femoral arterial segments of the whole blood clot (erythrocyte-rich) model before (Panel A) and after (Panel B) reperfusion. The whole blood clot is firmly anchored to the disrupted arterial wall and is morphologically very similar to the “head” region of coronary arterial thrombus.8 After reperfusion, the clot is reduced to some residual cells along the arterial wall. Figure 5 represents photomicrographs of the everted arterial graft (platelet-rich) model before (Panel A) and after (Panel B) reperfusion. The spontaneously formed thrombus is morphologically very similar to the “body” region of coronary arterial thrombus8 and is intimately attached to the adventitia. After reperfusion, residual platelet-rich clot is still evident. The morphologic characteristics of the arterial eversion model are further illustrated in Figure 6, which represents a light photomicrograph of a section obtained after occlusion stained with PTAH showing adventitial collagen fiber anchored into the platelet-rich thrombus.

Discussion

The aim of the present study was to determine whether or not there is a difference in sensitivity between erythrocyte-rich and platelet-rich arterial thrombus to lysis with rt-PA. Such differential sensitivity would support the hypothesis that the relative erythrocyte and platelet content of coronary arterial thrombus is a determinant for the efficacy of coronary artery reperfusion in patients with acute
myocardial infarction. Indeed, it is well established that coronary thrombus consists of both platelet-rich zones, mostly in physical contact with a fissured atheromatous plaque and of erythrocyte-rich areas extending proximally and distally to the site of plaque rupture, and of layers of platelet-rich and erythrocyte-rich material.

The present study, performed in rabbit models, shows that platelet-rich material occluding a femoral arterial occlusion graft is indeed much more resistant to lysis with intravenous or intra-arterial rt-PA than erythrocyte-rich whole blood clots. Indeed, intravenous infusion of rt-PA at a rate of 30 \( \mu \)g/kg/min, which results in plasma rt-PA levels of approximately 3 \( \mu \)g/ml, readily dissolved whole blood clots, whereas an infusion rate of 100 \( \mu \)g/kg/min, which was associated with a plasma rt-PA level of 22\( \pm \)5 \( \mu \)g/ml and extensive systemic fibrinogen breakdown, did not dissolve the platelet-rich thrombus in the arterial occlusion graft. However, our findings that platelet-rich occlusive material may be lyzed with local rt-PA infusion at a rate of 100 \( \mu \)g/kg/min indicates that the resistance to lysis is not absolute. These in vivo results confirm and extend the in vitro observation that whole blood clots are much more sensitive to lysis by rt-PA than platelet aggregates. The mechanism by which platelet-rich thrombus dissolves in vivo may be similar to that of in vitro platelets disaggregation as suggested by our findings that high local concentrations are required both to dissolve platelet-rich thrombus in vivo (infusion of 330 \( \mu \)l/min of rt-PA solution at a concentration of 0.75 mg/ml) and to disaggregate platelet clumps in vitro (100 \( \mu \)g/ml plasma). However, these high local rt-PA concentrations in vivo do not result in systemic alteration of ADP-induced aggregation. Reocclusion of the arterial occlusion graft after dissolution of the platelet-rich thrombus did not occur during continued infusion of rt-PA but was usually observed within 15 minutes after the end of the 1-hour rt-PA infusion. This may relate to the short half-life of the rt-PA, which results in rapidly decreasing plasma levels and may indicate that the maintenance of vessel patency requires either continued rt-PA infusion over longer time periods or interference with the mechanism of platelet deposition.

In our study, the dosages of 100 \( \mu \)g/kg/min of intra-arterial rt-PA required to reperfuse occluded everted graft segments only 6 mm long, were apparently much higher than the 1–2 \( \mu \)g/kg/min used by Hergrueter et al for the consistent reperfusion of everted grafts 1 cm long. Much of this difference, however, may be because we attempted to remove occlusive material formed in the absence of exogenous rt-PA infusion, whereas Hergrueter et al reperfused grafts occluded after termination of a 4-hour infusion of rt-PA. It is indeed well known that clots formed in the presence of low doses of rt-PA are much more susceptible to lysis than preformed clots, and this phenomenon may also apply to the platelet-rich occlusive material. This hypothesis would also explain that intravenous infusion of rt-PA at a dose sufficient to maintain the circulating blood level of rt-PA above 0.35 \( \mu \)g/ml effectively prevents short-term reocclusion in patients with acute myocardial infarction.

Most animal models for coronary thrombolysis have been developed with the use of whole blood clots in isolated segments of the coronary artery or thrombus induced by coagulation on a copper coil. Efforts to mimic coronary thrombosis have included the introduction of endothelial cell damage and of stenosis by external compression, but no model is available in which the thrombus consists of platelet-rich material or of distinct regions of platelet-rich and erythrocyte-rich thrombus. The present rabbit femoral arterial occlusion

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**Figure 3.** ADP-induced aggregation curves of platelet-rich plasma obtained in one representative experiment. I, aggregation induced with 18 \( \mu \)M ADP; II, aggregation induced with 2 \( \mu \)M ADP; A, baseline; B, after infusion of 20 \( \mu \)g/kg/min recombinant tissue-type plasminogen activator (rt-PA) for 25–30 minutes; C, after infusion of 80 \( \mu \)g/kg/min for 25–30 minutes; D, baseline plasma after addition of rt-PA to a final concentration of 5 \( \mu \)g/ml at the time of maximal aggregation; E, baseline plasma after addition of 100 \( \mu \)g/ml rt-PA.
Graft provides a model of arterial occlusion with predominantly platelet-rich material. Because of the nature of the thrombogenic stimulus (exposure of the collagen of adventitial tissue to flowing blood), we hypothesized that the mechanism of thrombosis in this model may have important similarities to the thrombotic occlusion after rupture of atheromatous plaque in patients with acute myocardial infarction. This is confirmed by the histologic findings of platelet-rich thrombus both in the arterial eversion graft and in the proximity of ruptured atheromatous plaque. Moreover, in three patients with acute myocardial infarction and coronary occlusions resistant to reperfusion with 1 mg/kg of intravenous rt-PA during 90 minutes, intracoronary administration of 0.5 mg/kg of rt-PA during 30 minutes caused reperfusion in two patients (unpublished observations). These preliminary results confirm that local infusion of rt-PA at a high rate may overcome resistance to intravenous rt-PA and are in agreement with the hypothesis that predominance of platelet-rich occlusive material may cause resistance to intravenous thrombolytic therapy.

In conjunction with the results of the present animal studies, these preliminary clinical observations in patients with acute myocardial infarction suggest that efforts to increase the efficacy of coronary thrombolysis to more than 75% should be directed toward the dissolution of platelet-rich thrombus. In this context, the rabbit femoral arterial eversion graft thrombosis model may constitute a useful tool for the investigation of such new thrombolytic agents and strategies.

Figure 4. Photomicrographs of whole blood clot thrombus model shows nonreperfused (Panel A) segment with a red cell thrombus (RCT) in the lumen that is attached to the disrupted arterial wall by fragments of media (arrows). The media (M) is thinned, but the adventitia (A) is intact. A reperfused arterial segment (Panel B) reveals residual red cell clot along the disrupted mural surface (arrows). (hematoxylin and eosin stain; original magnification, ×125)

Figure 5. Histogram of tissue sections from everted arterial segments show nonreperfused (Panel A) and reperfused (Panel B) vessels. The spontaneously occurring thrombus in this model is largely composed of platelet-rich clot (PT) that is intimately attached to the adventitia (A). The media (M) is intact. In Panel B, platelet-rich clot is still evident along the mural surface. (hematoxylin and eosin stain; original magnification, ×125)
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References


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Differential sensitivity of erythrocyte-rich and platelet-rich arterial thrombi to lysis with recombinant tissue-type plasminogen activator. A possible explanation for resistance to coronary thrombolysis.

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