Combination of Inhibition of Thrombin and Blockade of Thromboxane A2 Synthetase and Receptors Enhances Thrombolysis and Delays Reocclusion in Canine Coronary Arteries

Sheng-Kun Yao, MD; Judy C. Ober, BS; James J. Ferguson, MD; H. Vernon Anderson, MD; John Maraganore, PhD; L. Maximilian Buja, MD; and James T. Willerson, MD

Background. The efficacy of thrombolytic therapy in treating patients with acute myocardial infarction is limited by failure to achieve reperfusion in some patients, by the prolonged time required to achieve reperfusion, and by reocclusion of some coronary arteries. We designed this study to examine the effect of combined inhibition of thrombin and thromboxane synthesis and blockade of thromboxane A2 receptors in addition to tissue-type plasminogen activator (t-PA) on thrombolysis and reocclusion in an experimental canine model with coronary thrombosis.

Methods and Results. Blood flow velocity in the left anterior descending coronary artery (LAD) of 32 anesthetized mongrel dogs was monitored by a pulsed Doppler flow probe. Coronary thrombosis was induced by applying electrical stimulation to the LAD at the site where an external constrictor was used to narrow the artery. Three hours after the formation of occlusive thrombus, animals were randomly assigned to receive one of the following: 1) t-PA (80 μg/kg+8 μg · kg⁻¹ · min⁻¹ i.v.) and saline; 2) t-PA and hirulog, a hirudin-based synthetic peptide and specific thrombin inhibitor (2 mg/kg+2 mg · kg⁻¹ · hr⁻¹ i.v.); 3) t-PA and ridogrel, a combined thromboxane A2 synthetase inhibitor and receptor antagonist (5 mg/kg+2.5 mg · kg⁻¹ · hr⁻¹ i.v.); or 4) t-PA, hirulog, and ridogrel. Reperfusion developed in 14% (one of seven) of dogs treated with t-PA alone at an average of 86±4 minutes after treatment, in 78% (seven of nine) of dogs treated with t-PA plus hirulog at 53±11 minutes, in 13% (one of eight) of dogs treated with t-PA plus ridogrel at 85±5 minutes, and in 88% (seven of eight) of dogs treated with t-PA, hirulog, and ridogrel at 37±10 minutes (comparison of the frequency of and the time to reperfusion, both p<0.01). Among the dogs with reestablished coronary blood flow, reocclusion developed in the one treated with t-PA alone at 36 minutes after reperfusion, in seven of the seven treated with t-PA plus hirulog at 66±15 minutes, and in two of the seven treated with t-PA, hirulog, and ridogrel at 151±21 minutes (comparison of the frequency of and time to reocclusion, both p<0.05). Reocclusion was not detected in the one dog treated with t-PA and ridogrel or in the other five dogs treated with t-PA, hirulog, and ridogrel within 180 minutes after reperfusion. Hirulog prolonged and maintained activated clotting times at a level twice that of baseline values. Hirulog inhibited ex vivo platelet aggregation induced by thrombin, and ridogrel inhibited platelet aggregation induced by U46619, a thromboxane mimetic.

Conclusions. Inhibition of thrombin in addition to treatment with t-PA enhances thrombolysis. A combination of inhibition of thrombin and thromboxane synthetase and blockade of thromboxane A2 receptor enhances thrombolysis and delays or may prevent reocclusion of the recanalized coronary arteries. (Circulation 1992;86:1993–1999)

Key Words • platelet aggregation • thrombin • thrombolysis • thromboxane • tissue plasminogen activator

Thrombolytic therapy has become part of the standard treatment for patients with acute myocardial infarction. Recent clinical studies indicate that reperfusion of the ischemic myocardium preserves left ventricular function and reduces mortality.1–4 However, thrombolytic therapy is not always effective. In approximately 20% of patients, reperfusion is not achieved. The relatively prolonged time required to restore coronary blood flow after the administration of thrombolytic agents also reduces the efficacy of thrombolytic therapy. In addition, reocclusion of coronary arteries after reperfusion occurs in 10–20% of patients.5–9 Studies done in experimental animal models suggest that these limitations to thrombolytic therapy may be due to the activation of platelets and the release of platelet-derived factors in the coronary circulation.10,11

From the Cardiovascular Research Laboratory, Texas Heart Institute, Houston; the Departments of Internal Medicine, Pathology, and Laboratory Medicine, University of Texas Health Science Center at Houston; and Biogen, Cambridge, Mass.

Supported by National Heart, Lung, and Blood Institute Ischemic SCOR grant HL-17669.
Thromboxane A₂ and thrombin are important mediators of platelet activation.¹²⁻¹⁵ Upon release, thromboxane A₂ causes platelet aggregation and coronary artery constriction.¹²,¹³,¹⁶ Thrombin also activates platelets and participates in the formation of fibrin, which is another factor that contributes to the formation of an intracoronary thrombus.¹⁴,¹⁵,¹⁷ The activity of both thromboxane A₂ and thrombin increases after the administration of thrombolytic agents.¹⁸,¹⁹ Inhibition of thrombin or thromboxane A₂ may shorten the time required to achieve reperfusion and may prevent reoclusion of the recanalized coronary arteries.²⁰⁻²⁴

We designed this study to test the hypothesis that inhibition of thrombin combined with blockade of thromboxane A₂ synthetase and receptors enhances thrombolysis and delays coronary artery reocclusion. Hirulog, a hirudin-based synthetic peptide and specific thrombin inhibitor,²⁵ and ridogrel, a combined thromboxane A₂ synthetase inhibitor and receptor antagonist,²⁶,²⁷ were administered separately and together in addition to tissue plasminogen activator (t-PA) to dogs with experimentally induced intracoronary thrombosis to examine the effects of these agents on thrombolysis and coronary reocclusion.

**Methods**

All procedures used in this study were conducted according to the principles of the American Physiological Society and were approved by the Institutional Animal Care and Use Committee at the Texas Heart Institute, Houston.

**Surgical Preparation**

Mongrel dogs (n=32) weighing 25–35 kg were anesthetized intravenously with sodium pentobarbital (30 mg/kg), intubated, and placed on mechanical respirators (Harvard, model 60, Natick, Mass.). Plastic catheters were placed in a carotid artery for monitoring aortic pressure and in both a jugular and peripheral vein for administering drugs and fluids. A thoracotomy was performed through the left fifth intercostal space, and the heart was suspended in a pericardial cradle. A 1–2-cm segment of the left anterior descending coronary artery was carefully exposed, and nearby branches were ligated. An ultrasonic Doppler flow probe (Hartley Instruments, Houston, Tex.) was placed around the proximal portion of the exposed left anterior descending coronary artery to measure the velocity of blood flow. Baseline hemodynamics including heart rate, systolic and diastolic aortic pressure, and phasic and mean coronary blood flow velocity were recorded on an eight-channel recorder (model 3000, Gould, Inc., Cleveland, Ohio).

A needle electrode consisting of the tip (8 mm) of a 25-gauge needle crimped onto the end of a 30-gauge Teflon-insulated silver wire 10 cm in length was inserted at an oblique angle approximately 4 mm into the lumen of the exposed left anterior descending coronary artery at a site distal to the Doppler flow probe. The needle was stabilized on the vessel with 6-0 silk suture. To prevent leakage of current to the surrounding tissue, heat-shrink tubing was applied over the needle/wire and the soldered connection. A ground wire was connected to the subcutaneous tissue to complete the electrical circuit.

To induce thrombosis, an external constrictor was placed around the coronary artery at the needle insertion site to reduce arterial flow velocity by approximately 50%, and a current of 150 μA was applied through the electrode connected in series with the positive terminal of a 9-V battery, a 50 kΩ potentiometer, a multimeter, and the ground wire. Thrombotic occlusion was determined by a measure of zero flow velocity in the coronary artery. Electrical current was maintained for 30 minutes after persistent thrombotic occlusion had occurred.

**Experimental Procedures**

In this study, the intracoronary arterial thrombus was maintained for 3 hours, which approximates the amount of time that elapses between the onset of chest pain and the initiation of emergent assistance in most patients. Dogs were randomly assigned to receive one of the following drug regimens: 1) t-PA (Genentech, Inc., San Francisco, Calif.) and saline (group 1, n=7); 2) t-PA and hirulog (Biogen, Cambridge, Mass.) (group 2, n=9); 3) t-PA and ridogrel (Janssen, Beerse, Belgium) (group 3, n=8); or 4) t-PA, hirulog, and ridogrel (group 4, n=8). An intravenous bolus (80 μg/kg) of t-PA was administered, and a continuous intravenous infusion (8 μg·kg⁻¹·min⁻¹) was maintained for 90 minutes. In pilot studies with this model, we determined that this dosage of t-PA was the lowest effective dosage when administered with heparin at 200 units/kg. An intravenous bolus (2 mg/kg) of hirulog was administered, and a continuous intravenous infusion (2 mg·kg⁻¹·hr⁻¹) was maintained until the end of the study. An intravenous bolus (5 mg/kg) of ridogrel was administered, and a continuous intravenous infusion (2.5 mg·kg⁻¹·hr⁻¹) was maintained throughout the remainder of the study. We have previously reported that this dosage of ridogrel inhibits both in vivo and ex vivo platelet aggregation.²⁸,²⁹

We considered thrombolysis to have occurred when the velocity of flow in the coronary artery returned to at least 70% of the value before thrombus formation. Reperfusion time was defined as the time from t-PA administration to reperfusion. If reperfusion was not achieved after 90 minutes of t-PA infusion, the dogs were considered not to have had reperfusion and were excluded from further study. The reperfusion time in such dogs was counted as 90 minutes. Dogs in which reperfusion was achieved were monitored until the coronary arteries reoccluded or for a period of 180 minutes after reperfusion. Reocclusion time was defined as the time from reperfusion to reocclusion. Dogs without reocclusion after 180 minutes of reperfusion were considered not to have had reocclusion and reocclusion time was counted as 180 minutes. Dogs that developed reocclusion were monitored for an additional 30 minutes to document persistent reocclusion.

**Hematocrit, Coagulation, and Platelet Aggregation Studies**

Hematocrit was determined before and after termination of t-PA administration. Activated clotting time of whole blood was measured with the use of an automated blood coagulation timing device (HemoTec 2001370, Englewood, Colo.) before and at 5, 60, 120, and 180 minutes after the administration of t-PA.
Ex vivo platelet aggregation was analyzed before and 10 minutes after the administration of t-PA and hirulog or ridogrel in group 2 and group 3 dogs. Blood samples were collected in plastic tubes containing a 3.8% solution of sodium citrate (9 vol blood:1 vol sodium citrate). Platelet-rich plasma was obtained by centrifuging blood samples at 200g for 20 minutes, and platelet-poor plasma was obtained by centrifuging the residual blood at 3,000g for 10 minutes. The platelet count in platelet-rich plasma was adjusted to 300,000/mm³. A four-channel platelet aggregometer (Bio Data, model PAP4, Horsham, Pa.) was used for the assay. Platelet agonists used were U46619 (a thromboxane A₂ mimetic) at a final concentration of 40–160 ng/ml and thrombin at a final concentration of 0.25–0.75 units/ml. Because canine platelets do not aggregate in response to U46619 alone, epinephrine was added at 10 μM before the addition of U46619. The degree of platelet aggregation was reported as a percentage of maximal increase in light transmission in platelet-rich plasma as compared with platelet-poor plasma.

Statistical Analysis

All values are expressed as mean±SEM. Fisher’s exact test was used to compare the frequency of reperfusion and reocclusion in different groups of animals. A one-way ANOVA with repeated measurements was used to compare the hemodynamic values and activated clotting times obtained from different time periods. Student’s t test was used to compare the time to reperfusion and reocclusion in different groups of animals and the percentage of platelet aggregation before and after treatment in each group of animals. A probability value of less than 0.05 was considered significant.

Results

Oclusive thrombi developed in the coronary arteries of all 32 dogs within 10–30 minutes after the placement of external constrictors and induction of electrical stimulation. External constriction reduced phasic blood flow velocity in the coronary arteries to 61±8% of the baseline in group 1 dogs, to 56±5% in group 2 dogs, to 54±4% in group 3 dogs, and to 56±8% in group 4 dogs (p > 0.05, Table 1). Typical cyclic flow reductions associated with platelet aggregation were observed before total occlusion of the coronary arteries in most dogs (Figure 1). Slight but not significant changes in aortic pressures and heart rates were noticed after thrombus formation (Table 1).

Thrombolysis

Different drug regimens were administered to the four groups of dogs 3 hours after the occlusion of coronary arteries. Reperfusion was established after treatment with t-PA in only one of the seven dogs in group 1. In two of the other six dogs in group 1, only partial restoration of coronary flow occurred (<30% of the flow level recorded before thrombus formation); therefore, they were not included for further study. In seven of nine dogs in group 2, reperfusion was achieved after the administration of t-PA and hirulog. The restored coronary flow velocity in group 2 dogs was slightly higher than the flow velocity recorded before the formation of thrombus (Table 1). Only one of the seven dogs in group 3 had an acceptable level of reperfusion after treatment with t-PA and ridogrel. Coronary flow was reestablished in three other dogs in group 3 at a level <30% of the one recorded before thrombus formation. Coronary flow resumed in seven of eight dogs in group 4 after treatment with t-PA, hirulog, and ridogrel. The velocity of blood flow in group 4 dogs returned to a higher level than recorded before thrombus formation (Table 1). The frequency of reperfusion was significantly higher in groups 2 and 4 dogs than in groups 1 and 3 dogs (Figure 2A). The average time from drug treatment to lysis of thrombus was shorter in groups 2 and 4 dogs than in groups 1 and 3 dogs (Figure 2B). The difference between group 2 and group 4 was not statistically significant.

Reocclusion

Coronary reocclusion developed after reperfusion in the only dog in group 1 in which coronary blood flow was restored after treatment with t-PA. Coronary reocclusion occurred in all seven dogs from group 2 after reperfusion was achieved with t-PA and hirulog. Reocclusion was not observed within 180 minutes after recanalization of the coronary artery in the one dog in group 3 in which successful reperfusion was achieved by treatment with t-PA and ridogrel. Coronary reocclusion developed in only two of the seven dogs in group 4 with restored coronary blood flow after combined treatment with t-PA, hirulog, and ridogrel. In the other five dogs in group 4, coronary reocclusion did not develop within 180 minutes after reperfusion. The frequency of coronary reocclusion in group 4 dogs was significantly lower than that of the group 2 dogs (Figure 3A). In addition, the average time from reperfusion to reocclusion was significantly longer in group 4 dogs than in group 2 dogs (Figure 3B). Again, similar to that observed at the beginning of the initial thrombus formation, cyclic flow reductions in coronary arteries developed before total occlusion.

Hematocrit, Coagulation, and Platelet Aggregation Studies

Bleeding around the site of surgical incisions was not significant after treatment with t-PA alone; however, the addition of hirulog and/or ridogrel caused mild to moderate bleeding along the incision line. After treatment with t-PA, the hematocrit decreased slightly in dogs in groups 2, 3, and 4 but did not change in group 1 dogs (Table 2).

Treatment with t-PA or t-PA and ridogrel did not significantly affect activated clotting times in groups 1 and 3 (Figure 4). However, treatment with hirulog prolonged the activated clotting time to more than twice the baseline levels and maintained clotting times at the same levels for 3 hours in groups 2 and 4 (Figure 4).

As shown in Figure 5, ex vivo platelet aggregation induced by a thromboxane A₂ mimetic, U46619, was not altered by hirulog treatment but was significantly inhibited by ridogrel treatment. In contrast, thrombin-induced platelet aggregation was almost completely inhibited by hirulog treatment but was not affected by ridogrel treatment.

Discussion

In this study, we have demonstrated that combined treatment with hirulog, a hirudin-based specific throm-
TABLE 1. Hemodynamics Before and After Thrombolysis

<table>
<thead>
<tr>
<th>Time</th>
<th>Group 1 (t-PA+saline, n=7)</th>
<th>Group 2 (t-PA+hirulog, n=9)</th>
<th>Group 3 (t-PA+ridogrel, n=8)</th>
<th>Group 4 (t-PA+hirulog+ridogrel, n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heart rate (beats per minute)</td>
<td>Mean aortic pressure (mm Hg)</td>
<td>Flow velocity* (% of baseline)</td>
<td>Phasic Mean</td>
</tr>
<tr>
<td>Baseline</td>
<td>133±12</td>
<td>90±9</td>
<td>100 100</td>
<td></td>
</tr>
<tr>
<td>Stenosed</td>
<td>130±12</td>
<td>92±5</td>
<td>61±8 76±7</td>
<td></td>
</tr>
<tr>
<td>3 Hours after occlusion</td>
<td>119±10</td>
<td>92±7</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>90 Minutes after t-PA (n=6)</td>
<td>123±9</td>
<td>95±5</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>136±4</td>
<td>77±3</td>
<td>100 100</td>
<td></td>
</tr>
<tr>
<td>Stenosed</td>
<td>131±5</td>
<td>81±3</td>
<td>56±5 67±7</td>
<td></td>
</tr>
<tr>
<td>3 Hours after occlusion</td>
<td>117±5</td>
<td>93±3</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Reperfusion (n=7)</td>
<td>123±8</td>
<td>89±3</td>
<td>69±11 81±10</td>
<td></td>
</tr>
<tr>
<td>1 Hour after reperfusion (n=5)</td>
<td>115±15</td>
<td>84±7</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>139±10</td>
<td>80±6</td>
<td>100 100</td>
<td></td>
</tr>
<tr>
<td>Stenosed</td>
<td>137±9</td>
<td>86±5</td>
<td>57±4 70±7</td>
<td></td>
</tr>
<tr>
<td>3 Hours after occlusion</td>
<td>130±9</td>
<td>93±6</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>90 Minutes after t-PA (n=7)</td>
<td>126±11</td>
<td>70±3</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>136±6</td>
<td>82±4</td>
<td>100 100</td>
<td></td>
</tr>
<tr>
<td>Stenosed</td>
<td>135±6</td>
<td>86±3</td>
<td>56±8 65±3</td>
<td></td>
</tr>
<tr>
<td>3 Hours after occlusion</td>
<td>134±6</td>
<td>92±5</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Reperfusion (n=7)</td>
<td>124±6</td>
<td>86±6</td>
<td>72±5† 82±6†</td>
<td></td>
</tr>
<tr>
<td>1 Hour after reperfusion (n=5)</td>
<td>114±8‡</td>
<td>61±2‡</td>
<td>66±7 72±14</td>
<td></td>
</tr>
<tr>
<td>3 Hours after reperfusion</td>
<td>111±6‡</td>
<td>65±8‡</td>
<td>61±26 64±28</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM; t-PA, tissue-type plasminogen activator.

*Pertains to flow velocity in the coronary artery.

†P<0.05 compared with stenosed level.
‡P<0.05 compared with baseline level.

bin inhibitor, and t-PA enhances thrombolysis in dogs with coronary arterial thrombosis. In the same experimental model, treatment with hirulog, t-PA, and ridogrel, a combined thromboxane A2 synthetase inhibitor and receptor antagonist, reduces the frequency of reocclusion of recanalized coronary arteries.

Hirulog inhibits thrombin activity by occupying the anion-binding exosite and the catalytic site on the thrombin molecule.25 On a per-weight basis, hirulog is two times more active than hirudin in prolonging the activated partial thromboplastin time in human plasma.25 In our pilot studies, hirulog prolonged the activated clotting time in a dose-dependent manner. In the present study, hirulog prolonged activated clotting time to two times the baseline level and almost completely inhibited thrombin-induced ex vivo platelet aggregation. As a result, coronary thrombolysis was evident in seven of nine dogs treated with t-PA plus hirulog in contrast to only one of seven dogs treated with t-PA alone. The average time from treatment to reperfusion was also

**FIGURE 1.** Representative recording of coronary artery blood flow velocity after electrical stimulation to the endothelium. Upper panel: Phasic flow velocity; lower panel: mean flow velocity. Typical cyclic flow reductions caused by recurrent platelet aggregation and dislodgment were observed before complete occlusion of the artery. Paper speed, 0.25 mm/sec.
Thrombin, Thromboxane A₂, and Thrombolysis

Yao et al

FIGURE 2. Graphs illustrate frequency of reperfusion (panel A) and time to reperfusion (panel B) after drug treatments. Dogs in group 1 were treated with tissue-type plasminogen activator (t-PA) and saline; in group 2, with t-PA and hirulog, a thrombin inhibitor; in group 3, with t-PA and ridogrel, a combined thromboxane A₂ synthetase inhibitor and receptor antagonist; and in group 4, with t-PA, hirulog, and ridogrel. *Compared with groups 1 and 3, p<0.05.

significantly shorter in dogs treated with t-PA and hirulog than in dogs treated with t-PA alone. These data suggest that thrombin inhibition enhances t-PA-induced thrombolysis in coronary arteries.

After successful thrombolysis, recanalization of the reoccluded coronary arteries usually occurs, thus limiting the efficacy of thrombolysis. In this study, coronary reocclusion developed in all seven dogs treated with t-PA and hirulog despite continuous infusion of hirulog and maintenance of activated clotting times more than twice baseline levels. Similarly, previous clinical and experimental studies have shown that reocclusion occurs even when heparin is continued after reperfusion. These data suggest that thrombin inhibition may not prevent reocclusion. However, in previous studies, we have demonstrated that administering high dosages of heparin and maintaining activated clotting times four to five times baseline levels may delay or prevent coronary reocclusion. These findings suggest that hirulog may have failed to prevent reocclusion in this study because of insufficient antithrombin activity, especially at the arterial site of endothelial injury. This hypothesis is supported by the finding that thrombin levels are higher in patients with coronary reocclusion than in patients with patent coronary arteries after thrombolytic therapy. The fact that hirulog, at the dose we used in this study, enhanced thrombolysis but did not prevent reocclusion suggests that different levels of antithrombin activity are required for thrombolysis and reocclusion.

Ridogrel has two dose-dependent functions. At low doses, ridogrel significantly inhibits thromboxane A₂ synthesis. At high doses, as in the present study, ridogrel inhibits thromboxane A₂ synthesis and blocks thromboxane A₂/prostaglandin H₂ receptors. In this study, the inhibition of U46619-induced platelet aggregation after ridogrel treatment further demonstrates the antagonistic effect of ridogrel on thromboxane A₂/prostaglandin H₂ receptors.

Both actions of thromboxane A₂ synthesis inhibition and thromboxane A₂/prostaglandin H₂ receptor blockade strongly inhibit platelet aggregation. In addition, inhibition of thromboxane A₂ synthesis may redirect cyclic endoperoxides, which are the precursors of thromboxane synthesis, toward the synthesis of other prostaglandins. These prostaglandins, including prostacyclin and prostaglandin D₂, are inhibitors of platelet aggregation. By blocking thromboxane A₂/prostaglandin H₂ receptors, ridogrel prevents platelet activation by the accumulation of cyclic endoperoxide after the blockade of thromboxane synthesis. These effects make ridogrel a strong antiplatelet agent.

In previous studies in dogs with coronary artery stenosis and injured coronary endothelium, we have shown that ridogrel provides more protection against platelet accumulation than does the thromboxane A₂ synthetase inhibitor dazoxiben or the thromboxane A₂ receptor antagonist SQ29548. When administered with t-PA and heparin, ridogrel is also more effective in enhancing thrombolysis and delaying coronary reocclusion than either dazoxiben or SQ29548. In the present study, treatment with t-PA plus ridogrel resulted in reperfusion in only one of eight dogs—results that were not significantly different from those obtained in dogs treated with t-PA alone. Similar results have indicated that treatment with aspirin, another inhibitor of thromboxane synthesis, does not significantly accelerate thrombolysis. Together, these results suggest that simply blocking the effects of thromboxane may not significantly enhance thrombolysis in coronary arteries.

Treatment with t-PA, ridogrel, and hirulog significantly increased the frequency of successful thrombol-
ysis and decreased the time from treatment to thrombolysis when compared with administration of t-PA and hirulog. This finding suggests that inhibiting thromboxane synthesis, blocking thromboxane receptors, and, possibly, enhancing the production of antiplatelet prostaglandins such as prostacyclin may augment the effect of thrombin inhibition on enhancing thrombolysis induced by t-PA.

The rate of coronary reocclusion was significantly less in dogs treated with t-PA plus hirulog and ridogrel (two of seven dogs) than in dogs treated with t-PA and hirulog (seven of seven dogs). The average time from reperfusion to reocclusion was significantly longer in dogs treated with the combination of t-PA, hirulog, and ridogrel than in dogs treated with t-PA and hirulog. In the one dog in which coronary blood flow was reestablished after treatment with t-PA and ridogrel, reocclusion did not occur during the 180-minute follow-up period after recanalization of the artery.

In previous studies, administration of a single thromboxane A<sub>2</sub> receptor antagonist in addition to t-PA and heparin only slightly changed the time and frequency of coronary reocclusion. In this study, ridogrel, in addition to t-PA and hirulog, significantly delayed coronary reocclusion in most treated animals. This may be due to the combined effect of ridogrel on inhibiting thromboxane A<sub>2</sub> synthesis and blocking thromboxane A<sub>2</sub> receptors. Therefore, the addition of an agent like ridogrel to a regimen of t-PA and hirulog may be important in preventing coronary reocclusion.

Bleeding is usually a major complication of thrombolytic therapy. In this study, mild to moderate bleeding was observed around the surgical wound, particularly in animals treated with t-PA, hirulog, and ridogrel. Bleeding was associated with a modest reduction in hematocrit. The significant decrease in aortic blood pressure in animals treated with the combination of t-PA, hirulog, and ridogrel in part may be due to the anesthetic agents used in the study, because the heart rate also decreased significantly in this group of animals.

**Conclusions**

Thrombin inhibition is an important addition to t-PA in achieving successful thrombolysis. Blockade of thromboxane A<sub>2</sub> synthetase and receptors may help delay or prevent coronary reocclusion after successful thrombolysis. When a thrombin inhibitor and a thromboxane A<sub>2</sub> synthetase inhibitor and receptor antagonist are used together, the t-PA–induced thrombolysis is enhanced, and coronary reocclusion is delayed or may be prevented in some cases.

**Acknowledgment**

We thank Rebecca Bartow, PhD, for editorial consultation in the preparation of the manuscript.

**References**


Combination of inhibition of thrombin and blockade of thromboxane A2 synthetase and receptors enhances thrombolysis and delays reocclusion in canine coronary arteries.

S K Yao, J C Ober, J J Ferguson, H V Anderson, J Maraganore, L M Buja and J T Willerson

doi: 10.1161/01.CIR.86.6.1993

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/86/6/1993