Kistrin, a Polypeptide Platelet GPIIb/IIIa Receptor Antagonist, Enhances and Sustains Coronary Arterial Thrombolysis With Recombinant Tissue-Type Plasminogen Activator in a Canine Preparation

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Background. Kistrin is a 68-amino acid polypeptide from the venom of the Malayan pit viper Agkistrodon rhodostoma, which inhibits the platelet GPIIb/IIIa receptor. Its effect on thrombolysis, reocclusion, and bleeding associated with administration of recombinant tissue-type plasminogen activator (rt-PA) was studied in a canine model of coronary artery thrombosis.

Methods and Results. Coronary patency was monitored for 2 hours by ultrasonic flow probe and repeated coronary angiography. The rt-PA was given as 0.45-mg/kg bolus injections at 15-minute intervals until recanalization or to a maximum of four boluses. Four groups of four or five dogs were studied: a control group that received intravenous heparin (4,000-unit bolus and 1,000 units each hour) and three groups that received heparin and 0.48, 0.24, or 0.12 mg/kg kistrin, administered as a 10% bolus injection and an infusion during a 60-minute period. In the control group, reflow occurred in four of five dogs within 37±47 minutes but was followed by cyclic reflow and reocclusion. Kistrin at a dose of 0.48 and 0.24 mg/kg reduced the time to reflow to 6±5 and 10±3 minutes, respectively, and abolished reocclusion. With 0.12 mg/kg kistrin, reflow occurred in all four animals, within 27±23 minutes, and reocclusion occurred in two animals. Kistrin induced a dose-related prolongation of the template bleeding time: with 0.48 mg/kg kistrin, the bleeding time was prolonged from 3.8±1.3 minutes before infusion to 29±2 minutes during infusion, but it was shortened to 8.3±2.6 minutes at 90 minutes after the end of infusion. Kistrin also caused a dose-related inhibition of platelet aggregation with ADP and collagen: with 0.48 mg/kg kistrin, platelet aggregation was abolished during the infusion but had partially recovered toward the end of the observation period. Pathological examination of recanalized coronary arterial segments of dogs given 0.48 or 0.24 mg/kg kistrin revealed widely patent arteries with some platelets layered on the damaged intimal surface.

Conclusions. Kistrin increases the rate and extent of thrombolysis with a reduced dose of rt-PA, and it prevents reocclusion. At an effective dose, it is associated with a transient prolongation of the bleeding time and inhibition of platelet aggregation. Kistrin may offer promise as adjunctive treatment to thrombolytic agents in patients with acute myocardial infarction. (Circulation 1991;83:1038–1047)

Thrombolytic therapy of acute myocardial infarction has become an established procedure for reperfusion of occluded coronary arteries, resulting in reduction of infarct size and preservation of left ventricular function.1 Despite their widespread use, the currently available thrombolytic agents are associated with significant risk of reocclusion, which may be avoided by the use of kistrin.2

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bolytic agents and strategies suffer from a number of significant limitations, including resistance to reperfusion in approximately 25% of patients,\textsuperscript{2,3} occurrence of acute coronary reocclusion in 5–25%,\textsuperscript{4,5} a prolonged time to restoration of antegrade coronary flow of an average of 45 minutes,\textsuperscript{6–8} and a bleeding tendency with a frequency of intracerebral bleeding of approximately 0.5%.\textsuperscript{9–11} Various approaches to overcome the present limitations of thrombolytic therapy are currently under investigation in laboratory animal models.

Platelet-mediated thrombosis is a major pathogenic mechanism underlying the limited efficacy of thrombolytic therapy.\textsuperscript{12–16} Several approaches have been investigated to prevent platelet-mediated coronary occlusion including the use of aspirin,\textsuperscript{17} prostaglandin E\textsubscript{1},\textsuperscript{18} selective thromboxane A\textsubscript{2} synthase inhibitors,\textsuperscript{19} selective thromboxane A\textsubscript{2} receptor antagonists in combination with serotonin receptor antagonists,\textsuperscript{20,21} combined thromboxane A\textsubscript{2} synthase inhibitors/prostaglandin endoperoxide receptor antagonists,\textsuperscript{22} or selective thrombin inhibitors.\textsuperscript{23–26}

The platelet receptor GPIIb/IIIa mediates platelet aggregation by most physiological agonists\textsuperscript{27} and, therefore, may constitute a preferred target for therapeutic intervention in thromboembolic disease. The GPIIb/IIIa receptor may be inhibited by several compounds, including monoclonal antibodies\textsuperscript{28} that exhibit potent antithrombotic effects in vivo\textsuperscript{29–32} but also by peptides derived from the gamma chain of fibrinogen\textsuperscript{33} or Arg-Gly-Asp-containing (RGD) peptides\textsuperscript{34,35} that prevent thrombosis in vivo.\textsuperscript{36–40} Kistrin is a polypeptide (\(M_r\), 7,334) composed of 68 amino acids, isolated from the venom of the Malayan pit viper Agkistrodon rhodostoma, which contains the typical RGD recognition sequence.\textsuperscript{41} It belongs to a family of proteins from pit viper venoms that are specific human platelet GPIIb/IIIa receptor antagonists that inhibit platelet aggregation.\textsuperscript{42–45} Kistrin is 100–1,000 times more potent than the pentapeptide GRGDS for the inhibition of fibrinogen binding to platelets and for platelet aggregation, and its binding to platelets is both rapid and fully reversible.\textsuperscript{41}

In the present study, we evaluated the effects of adjunctive use of kistrin with recombinant tissue-type plasminogen activator (rt-PA), in a canine model of coronary artery thrombosis with superimposed high grade stenosis, on: 1) the rate and extent of thrombolysis, 2) the frequency of reocclusion, 3) prolongation of the bleeding time, and 4) inhibition of ex vivo platelet aggregation.\textsuperscript{46}

Methods

Reagents

The single-chain rt-PA used was Activase, supplied by Genentech, Inc., South San Francisco, Calif. Kistrin was purified from the venom of Agkistrodon rhodostoma and characterized as described elsewhere,\textsuperscript{41} and was also supplied by Genentech. Heparin was purchased from Sinn, Cherry Hill, N.J.

Canine Left Anterior Descending Coronary Arterial Thrombosis

The experimental model was used essentially as previously described.\textsuperscript{46} Adult mongrel dogs (20–25 kg) were anesthetized with pentobarbital (30 mg/kg body wt i.v. and with additional doses as required). The dogs were intubated and were placed on a respirator with a tidal volume of 10–15 ml/kg. Procarbazine, 1.5 g i.m., and lidocaine, 0.1 mg/kg/min i.v., were given for prophylaxis of arrhythmias. The left carotid artery was exposed through an incision in the neck and was cannulated with a 7-1 modified Amplatz Judkins coronary angiographic catheter. Thoracotomy was performed through the left fifth intercostal space, with cannulation of the left internal mammary artery for continuous blood pressure recording. The pericardium was opened and was suspended to create a pericardial cradle. The left anterior descending coronary artery was dissected, and a 2.5-cm segment was isolated distal to the first diagonal branch. A 0.7-mm internal diameter catheter was inserted into a side branch of the isolated left anterior descending coronary artery segment, and an ultrasonic flow probe (T101 Transsonic System Inc., Ithaca, N.Y.) was placed on the proximal portion of the artery for continuous blood flow monitoring. Selective angiography of the left anterior descending coronary artery was obtained with 1–2 ml meglumine diatrizoate with videotape recording. One milliliter of blood was drawn for thrombus formation.

The dog was then given heparin intravenously (bolus of 4,000 units and 1,000 units at hourly intervals). A 2-mm wide plastic wire tie (Mass Gas and Electric Supply, Watertown, Mass.) was progressively constricted around the left anterior descending coronary artery, just distal to the proposed site of thrombus formation, to limit the blood flow to 40±10% of baseline. A control angiogram was then obtained.

The isolated left anterior descending coronary artery segment was traumatized by four consecutive external compressions with blunt forceps during 3–5 seconds. This procedure was performed to damage the endothelium and, thereby, to promote thrombus adherence. Snare occlusions were made distal to the flow probe and proximal to the constriction site. Thrombin, 0.1 ml of 100 units/ml (Thrombina, Armour Pharmaceutical, Kankakee, Ill.) mixed with 0.3 ml blood was injected through the side branch catheter into the emptied coronary artery segment. After 5 minutes, the proximal snare was released; 2 minutes later, the distal tourniquet was released. An angiogram was obtained 30 minutes after thrombus formation to confirm total occlusion of the artery as demonstrated by the ultrasonic flow probe.

Infusion Protocols and Evaluation of Coronary Artery Patency

Four treatment groups were studied: 1) a control group given heparin alone; 2) three groups given
heparin and an intravenous infusion of kistrin at a
dose of 0.48, 0.24, or 0.12 mg/kg, which was given as
a bolus of 10% of the total dose followed by infusion
of the remainder during a 60-minute period (rate of
8, 4, or 2 μg/kg/min, respectively). Intravenous infu-
sion was performed with a constant-rate infusion
pump (Harvard Apparatus, South Natick, Mass.),
starting after stable complete occlusion for 30 min-
utes had been confirmed by coronary angiography.
Fifteen minutes later, rt-PA was given as an intrave-
nous bolus injection of 0.45 mg/kg at 15-minute
intervals until recanalization of the thrombosed cor-
onary artery was achieved, or to a maximum of four
boluses. Angiograms were obtained every 15 minutes
to monitor occlusion and additionally when the flow
probe showed evidence of reflow. Reflow was moni-
tored by angiography and flow probe for at least 2
hours after the initial angiographic confirmation of
stable coronary artery occlusion. The animals were
killed, on average, 150 minutes after the start of the
kistrin infusion.

The reflow time was recorded as the time from the
first rt-PA bolus injection until recanalization was
documented by the return of blood flow in the artery
to at least 25% of that before thrombus formation
and was confirmed by complete angiographic filling
of the apex with rapid clearance of the dye in four
heart beats or less. After recanalization was achieved,
blood flow was monitored for evidence of reocclusion,
defined as less than 25% of baseline flow; the final
confirmation was obtained by angiography that
showed dye clearance in more than five cycles. The
reocclusion time was defined as the interval between
documented reflow and reocclusion. Frequently, cyclic
reflow occurred, which was interspersed with periods
of reocclusion.

The coronary artery patency status was categorized
as follows: 1) persistent occlusion: no reflow; 2) cyclic
reflow and reocclusion: alternating reocclusion and
recanalization after initial reflow; 3) persistent patency:
persistent flow without reocclusion after initial reflow.

Blood Analyses

Venous blood samples for platelet and plasma assays
were collected in 0.01 M citrate and 200 kallikrein
inhibitor units (KIU) aprotinin/ml (Sigma Chemical
Co., St. Louis, Mo.). Platelet-rich plasma was prepared
immediately and was tested for platelet aggregation
induced with 11 μM ADP or 0.1 mg/ml collagen
(Sigma). Plasma was prepared from blood kept on ice
until the end of the experiment; then, it was centrifuged
at room temperature and was stored at −20°C. Fibrin-
ogen was measured by a coagulation rate assay\textsuperscript{47,48} that is insensitive to therapeutic concentrations of hepa-
arin\textsuperscript{49} and t-PA antigen was measured with a mono-
clonal antibody–based enzyme-linked immunosorbent
assay (ELISA)\textsuperscript{50} that does not cross-react with canine
t-PA. Venous blood samples collected in EDTA were
also obtained for measurement of the platelet count.
Bleeding times were measured before the first injec-
tion, 20 minutes after the first injection, and 60 minutes
after the last injection of rt-PA; measurements were
made with a spring-loaded blade device (Surgicutt
International, Technidyne Corp., Edison, N.J.) applied
to a shaved foreleg. Kistrin levels in plasma were assayed
by specific ELISA.

Pathological Examination

At the end of the experiment, the dogs were killed
with an overdose of pentobarbital. The heart was
removed; persistently occluded and reoccluded arter-
ies were perfusion fixed with 5% buffered formalin in
situ\textsuperscript{51} and the whole heart was fixed in 5% formal-
dehyde. The thrombosed, stenotic, and poststenotic
segments of the left anterior descending coronary
artery with contiguous 0.5 cm of proximal artery and
the distal stenosis were then removed intact, were
embedded in paraffin blocks, and were sectioned
longitudinally. Sections were stained with hematoxy-
lin and eosin and were examined microscopically for
the presence of intraluminal or mural thrombi. Per-
sistent patent arteries were subjected to perfusion
fixation with 0.1 M cacodylate–buffered 2.5% glu-
taraldehyde for scanning electron microscopy as previ-
ously described.\textsuperscript{25,51} The extent of thrombosis was
semi-quantitatively graded on a scale of 1 to 4: 1, no
or minimal mural thrombus; 2, mural thrombus occup-
ying less than 50% of the luminal diameter over a
section greater than the diameter of the arterial
segment; 3, thrombus occupying more than 50% but
less than 95% of the luminal diameter; and 4, com-
pletely occlusive thrombus or occupying more than
95% of the luminal diameter. The composition of the
thrombus was characterized as erythrocyte-rich,
platelet-rich, or mixed with interlaced platelet-rich
and erythrocyte-rich zones.

Statistical Analysis

The values are reported as mean±SD. The signifi-
cance of differences between groups was determined
with Student’s \textit{t} test for paired or unpaired values.
Fisher’s exact test was used to compare the occur-
rence of reflow and reocclusion in the various groups.
A Kruskal-Wallis nonparametric analysis of variance
was performed\textsuperscript{52} on ranks of the ordered variable
of arterial patency, which ranges from 0 (persistent
occlusion) to 1 (cyclic reflow and reocclusion), and to
2 (persistent patency, as determined with the blood
flow meter). A similar analysis was performed on
arterial patency, graded on the pathological analysis
as described above. This form of analysis of variance
was selected because of the non-Gaussian distribu-
tion of the patency-state variables.

Results

Coronary Artery Reflow and Reocclusion

The results of coronary blood flow and coronary
artery patency status, categorized as persistent occlu-
sion, cyclic reflow and reocclusion, and persistent
patency, as defined in “Methods,” are summarized in
Table 1. The patency status in the individual animals
is also schematically represented in Figure 1. The external constrictor reduced the blood flow in the different groups on average to 39–46% of baseline, from a mean value of 11–16 ml/min to 5–6.6 ml/min before thrombus formation (Table 1).

Bolus injection of rt-PA in control animals without kistrin induced recanalization in four of five animals (Figure 1). Reflow was achieved within 37±47 minutes, with a median time of 25 minutes and a range of 3–120 or more minutes (Table 2). Cyclic reflow and recanalization occurred in all four dogs. The median number of rt-PA boluses administered was two, with a range of one to four. Bolus injection of rt-PA induced recanalization in all five animals given 0.48 mg/kg kistrin (Figure 1). Reflow was achieved in all animals with a single bolus of rt-PA, with a median time of 6 minutes and a range of 3–15 minutes (Table 2). Reocclusion did not occur, except for one brief cycle of reocclusion and reflow in one dog. A single bolus of rt-PA, combined with 0.24 mg/kg kistrin, induced reflow in all five dogs, with a median time of 10 minutes and a range of 7–15 minutes. Brief periods of reocclusion occurred in two of these five dogs. With 0.12 mg/kg kistrin, reperfusion required more than one rt-PA bolus in two of four animals in which reflow was followed by cyclic reflow and reocclusion. The median number of rt-PA boluses was two, with a range of one to four.

Several significant differences in coronary arterial patency were observed with the various infusions.

### Table 1. Results of Coronary Blood Flow and Coronary Artery Patency Status

| Agent   | Dose (mg/kg) | n  | Blood flow (ml/min) | Coronary patency status (frequency) | | | |
|---------|--------------|----|---------------------|------------------------------------|---|---|
|         |              |    | Baseline            | Poststenotic | Postreflow | PO | CR | PP |
| Control | —            | 5  | 13±2.4              | 5.1±0.9    | 3.6±1.0    | 1  | 4  | 0  |
| Kistrin | 0.48         | 5  | 11±0.9              | 4.9±1.4    | 3.2±1.2    | 0  | 1  | 4  |
|         | 0.24         | 5  | 16±1.4              | 6.6±1.0    | 2.5±0.4    | 0  | 2  | 3  |
|         | 0.12         | 4  | 11±1.1              | 5.0±0.8    | 2.3±0.5    | 0  | 2  | 2  |

Data are mean±SD.

PO, persistent occlusion; CR, cyclic reflow; PP, persistent patency.

### Table 2. Effects of Kistrin on Time to Left Anterior Descending Coronary Artery Recanalization and Number of rt-PA Boluses Required to Achieve Recanalization

| Agent   | Dose (mg/kg) | n/total n | Reperfusion | rt-PA boluses (n) | | | |
|---------|--------------|-----------|-------------|------------------|---|---|
|         |              |           | Time (min)  |                  | Median | Range |
| Control | —            | 4/5       | 37±47       | 25               | 3–120 | 2 1–4 |
| Kistrin | 0.48         | 5/5       | 6±5         | 6                | 3–15  | 1 1–1 |
|         | 0.24         | 5/5       | 10±3        | 10               | 7–15  | 1 1–1 |
|         | 0.12         | 4/4       | 27±23       | 21               | 7–55  | 2 1–4 |

Data are mean±SD of values in responsive animals, and the median and range are values for the total group.

rt-PA, recombinant tissue-type plasminogen activator.

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**Figure 1. Schematic representation of the left anterior descending coronary arterial patency status in dogs receiving 0.45-mg/kg bolus injections of recombinant tissue-type plasminogen activator (rt-PA) and kistrin. ▼, rt-PA bolus injection; □, continuous intravenous infusion of kistrin. Open bars, patency; solid bars, occlusion of the coronary artery.**
protocols. A Kruskal-Wallis analysis of all experiments with patency status ordered in the sequence of persistent occlusion, cyclic reflow and reocclusion, and persistent patency yielded a p value of 0.01. Comparison of the patency status in the group with heparin and 0.48 mg/kg kistrin with that of the control group given heparin alone gave a p value of 0.006. Persistent patency occurred more frequently with the combination of kistrin and heparin than with heparin alone (five of five versus zero of five dogs, respectively, p=0.008 by Fisher's exact tests).

The time to reflow was significantly shorter in the groups receiving 0.48 mg/kg kistrin than in the group receiving heparin alone (p=0.05). Likewise, these groups receiving kistrin (0.48 or 0.24 mg/kg) achieved reperfusion with fewer rt-PA boluses than did the group given heparin alone (p=0.02).

**Hemostasis Analysis**

Serial template bleeding times were measured in all animals with results summarized in Table 3. In control animals given heparin and rt-PA, the template bleeding time did not change significantly throughout the experiment. Kistrin in combination with rt-PA prolonged the bleeding time: with 0.48-mg/kg kistrin, bleeding time was prolonged from 3.8±1.3 minutes before injection to 29±2.2 minutes within 20 minutes after the first rt-PA bolus injection (30 minutes into the kistrin infusion); with 0.24 mg/kg kistrin, the bleeding time was prolonged from 3.9±1.3 minutes before infusion to 18±11 minutes during infusion; and with 0.12 mg/kg of kistrin, bleeding time was prolonged from 3.0±0.4 minutes before infusion to 6.0±1.1 minutes during infusion. The bleeding time prolongation was, however, rapidly reversible, for near normalization occurred within 90 minutes after the end of the kistrin infusion (at the end of the experiment) (Table 3).

Table 4 summarizes the results of platelet function tests. Platelet counts did not markedly decrease except moderately at the highest dose of kistrin. ADP-induced and collagen-induced platelet aggregation were impaired to an extent proportional to the kistrin dose, with partial recovery toward the end of the experiment. In the control group, platelet aggregation remained essentially unaltered throughout the experiment.

Results of plasma kistrin levels, rt-PA antigen and fibrinogen levels, and of hematocrit determination are summarized in Table 5. Infusion of 0.48 mg/kg kistrin resulted in a steady-state plasma level of 0.51±0.19 μg/ml. This was followed by rapid clearance of kistrin from plasma to reach near-background levels toward the end of the experiment. Plasma rt-PA levels increased to 3–4.5 μg/ml, measured 1 minute after bolus injection, and decreased to 0.3–0.5 μg/ml within 10 minutes. The fibrinogen level in the different groups of animals decreased moderately but remained greater than 50% of baseline in all groups. The hematocrit value decreased moderately (<30%) in all groups.

**Pathology**

The results of pathological analysis are summarized in Table 6. The extent of arterial thrombosis was graded, and the thrombus composition was characterized as described in “Methods” and in the legend of Table 6. Segments not recanalized primarily contained occlusive red cell clots (Figure 2); segments with reocclusion after initial reflow contained occlusive thrombi composed of red cell and platelet-rich clots; and segments with persistent pa-
TABLE 5. Results of Blood Analyses

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/kg)</th>
<th>Plasma kistrin level (µg/kg)</th>
<th>rt-PA antigen level (µg/ml)</th>
<th>Fibrinogen level (g/l)</th>
<th>Hematocrit</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td>40 min</td>
<td>End</td>
<td>1 min after rt-PA</td>
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<tr>
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<td>--</td>
<td>--</td>
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<td>Kistrin 0.48</td>
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<td>0.25±0.15</td>
<td>0.51±0.19</td>
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<td>4.6±1.2</td>
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<tr>
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<td>--</td>
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<td>3.1±1.2</td>
</tr>
</tbody>
</table>

Data are mean±SD.
rt-PA, recombinant tissue-type plasminogen activator.
The background value measured in plasma before kistrin infusion is 0.05±0.04 µg/ml.

Platelet aggregation with the potent monoclonal antiplatelet GPIIb/IIIa antibody 7E3 overcomes resistance of platelet-rich thrombus to thrombolysis and accelerates and sustains coronary artery reperfusion with reduced doses of rt-PA. However, in the setting of high-grade stenosis and endothelial damage, these effects are only obtained at extensive saturation of platelet GPIIb/IIIa receptors, resulting in protracted inhibition of platelet aggregation and prolongation of the bleeding time.

Many peptides and proteins, derived from the RGD sequence or the gamma-chain recognition sequences of fibrinogen, have binding affinity for the GPIIb/IIIa receptor. These compounds compete with fibrinogen binding to the GPIIb/IIIa receptor, which constitutes a common final pathway in platelet aggregation.

TABLE 6. Pathological Analysis of Left Anterior Descending Coronary Arterial Segments

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/kg)</th>
<th>Dog number</th>
<th>Analysis</th>
<th>Patency status</th>
<th>Description</th>
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<tbody>
<tr>
<td>Control</td>
<td>--</td>
<td>1</td>
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<td>ER</td>
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<td>2</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>SEM</td>
<td>1</td>
<td>Platelet layer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>HE</td>
<td>4</td>
<td>PR</td>
<td></td>
</tr>
<tr>
<td>Kistrin 0.48</td>
<td>1</td>
<td>SEM</td>
<td>1</td>
<td>Platelet monolayer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>SEM</td>
<td>1</td>
<td>Platelet layer, some fibrin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>SEM</td>
<td>1</td>
<td>Platelet layer</td>
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<tr>
<td></td>
<td>4</td>
<td>SEM</td>
<td>1</td>
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<td></td>
<td>2</td>
<td>SEM</td>
<td>2</td>
<td>PR at stenosis</td>
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<td>3</td>
<td>SEM</td>
<td>1</td>
<td>Some platelets</td>
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<tr>
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<td>SEM/HE</td>
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<td>ER</td>
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<td>SEM</td>
<td>2</td>
<td>PR</td>
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<tr>
<td></td>
<td>3</td>
<td>SEM</td>
<td>1</td>
<td>MPE</td>
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<td>4</td>
<td>SEM</td>
<td>1</td>
<td>Platelet monolayer</td>
<td></td>
</tr>
</tbody>
</table>

SEM, analysis by scanning electron microscopy; HE, analysis by histologic analysis after hematoxylin and eosin staining; ER, erythrocyte-rich thrombus; PR, platelet-rich thrombus; MPE, mixed thrombus with interlaced platelet-rich and erythrocyte-rich zones.
Patency status: 1, no or minimal mural thrombus; 2, mural thrombus occupying less than 50% of the luminal diameter over a section greater than the diameter of the arterial segment; 3, thrombus occupying more than 50% but less than 95% of the luminal diameter; and 4, completely occlusive thrombus or occupying more than 95% of the luminal diameter.
aggregation. Several RGD-containing snake venom polypeptides inhibit fibrinogen binding and platelet aggregation 100–1,000 times more efficiently than does the GRGDS pentapeptide.\textsuperscript{39,41} One of these polypeptides, kistrin, obtained from the venom of \textit{Agkistrodon rhodostoma} was recently characterized.\textsuperscript{41} It binds with a high affinity (\(K_d\) in the nanomolar range) to both resting and ADP-activated human platelets, and it inhibits ex vivo platelet aggregation reversibly after in vivo injection in rabbits.

We have used a canine model of coronary artery thrombosis with superimposed high-grade stenosis, which has anatomical similarities to the coronary artery occlusion in patients with acute myocardial infarction,\textsuperscript{46} to study the effects of kistrin on the efficacy and speed of thrombolysis with rt-PA, on reocclusion, on the bleeding tendency as revealed by the template bleeding time, and on ex vivo platelet aggregation. Simultaneous systemic therapeutic heparin anticoagulation was used because it is mandatory in achieving the full effect of the monoclonal GPIIb/IIIa antibody\textsuperscript{56} (and unpublished observations).

Intravenous infusion of kistrin at a dose of 0.48 or 0.24 mg/kg infused during 1 hour, which prolonged the template bleeding time from approximately 4 to 20 minutes or more and which abolished ADP- and collagen-induced ex vivo platelet aggregation, markedly accelerated coronary arterial thrombolysis with negligible residual thrombus demonstrated on pathological examination. These observations are in agree-

![Figure 2. Scanning electron micrograph of a left anterior descending coronary arterial segment from a dog treated with heparin and recombinant tissue-type plasminogen activator (rt-PA), which resulted in cyclic reflow. Thrombus is erythrocyte-rich clot. Original magnification, \(\times 1,900\).](image)

![Figure 3. Scanning electron micrograph of a persistently patent left anterior descending coronary artery segment in a dog given one bolus of 0.45 mg/kg of recombinant tissue-type plasminogen activator with 0.48 mg/kg kistrin. Intimal surface reveals numerous platelets and a few red cells but no fibrin strands. Original magnification, \(\times 2,200\).](image)
ment with the much higher affinity of kistrin for the platelet GPIIb/IIIa receptor \(^4\) compared with tetrapeptides such as RGDY. \(^5\) Indeed, the effective dose of 4 \(\mu g/\text{kg/min}\) to prevent reocclusion is 1,000 times lower than that of the tetrapeptide. \(^7\)

Reocclusion was abolished during the 1-hour follow-up period after the infusion of kistrin, during which the prolongation of the bleeding time and the inhibition of platelet aggregation returned toward normal. The persistence of coronary patency, despite the near normalization of the bleeding time, suggests either that the time required for passivation of the damaged endothelial surface is relatively short or that the extensive dissolution of the occluding thrombus by the combination of rt-PA, heparin, and kistrin resulted in a nonthrombogenic vessel segment. Indeed, residual thrombus has been shown to be a more potent thrombogenic stimulus than a damaged vessel wall. \(^8\)

In the present study, we administered kistrin as a loading dose of 10% followed by a continuous intravenous infusion at a rate of 2, 4, or 8 \(\mu g/\text{kg/min}\) for 60 minutes. In preliminary experiments (not shown), this dose range was determined to result in partial-to-complete inhibition of platelet aggregation and in marginal-to-extensive prolongation of the bleeding time. The infusion of kistrin was started 15 minutes before the bolus injections of rt-PA to allow evaluation of its effect on time to recanalization under steady-state plasma concentrations. The dose of heparin was selected to maintain the “activated” partial thromboplastin time more than twofold throughout the experimental observation period. Insufficient heparin anticoagulation, indeed, reduces the ability of rt-PA to recanalize occluded arteries, and adequate heparinization is required for sustained patency after the administration of GPIIb/IIIa antagonist (unpublished observations).

Thus, acceleration of lysis and prevention of reocclusion with the combination of a reduced dose of rt-PA, heparin, and a short-lived reversible platelet GPIIb/IIIa antagonist can be accomplished with transient prolongation of the bleeding time, possibly resulting in a less-increased risk of bleeding than would have occurred with protracted platelet inhibition. However, potent combined antiplatelet, anticoagulant, and thrombolytic therapy, as evaluated in this experimental animal study, is expected to be inherently associated with some increased bleeding risk, which will have to be carefully documented in humans. Provided that the observations of the present study can be extrapolated to humans, this combination therapy may result in improved efficacy to toxicity ratios for pharmacological recanalization of occluded coronary arteries in patients with acute myocardial infarction.

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