Intravenous and Endobronchial Administration of G4120, a Cyclic Arg-Gly-Asp–Containing Platelet GPIIb/IIIa Receptor–Blocking Pentapeptide, Enhances and Sustains Coronary Arterial Thrombolysis With rt-PA in a Canine Preparation

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G4120, L-cysteine, \(N\)-(mercaptoacetyl)-D-tyrosyl-L-arginylglycyl-L-\(\alpha\)-aspartyl-cyclic(\(1\rightarrow5\))-sulfide, 5-oxide, a synthetic cyclic Arg-Gly-Asp–containing pentapeptide, has a high affinity (dissociation constant of 4 nM) for the platelet glycoprotein (GP) IIb/IIIa receptor. The effects of its intravenous or endobronchial administration on thrombolysis, reocclusion, and bleeding time prolongation induced with 0.45 mg/kg bolus injections of recombinant tissue-type plasminogen activator in combination with intravenous heparin (4,000-unit bolus and 1,000 units each hour) were studied in a canine model consisting of an erythrocyte-rich blood clot in the left anterior descending coronary artery. Coronary patency was monitored for 3 hours both by ultrasonic flow probe and by repeat coronary angiography. Four groups of six to 10 dogs were studied with intravenous infusions of 0, 0.1, 0.2, or 0.3 mg/kg G4120 over 60 minutes. G4120 at a dose of 0.3 mg/kg reduced the time to reflow from a mean control value of 45 to 8 minutes (p=0.036) and delayed reocclusion (p=0.001). Four groups of five or six dogs were studied with endobronchial instillation of G4120 in a randomized, blinded study design using 0, 0.13, 0.25, or 0.5 mg/kg G4120. Endobronchial G4120 at a dose of 0.5 mg/kg reduced the time to reflow from a mean control value of 52 to 7 minutes (p=0.039) and abolished cyclic reocclusion and reflow (p=0.008). G4120 induced a dose-related transient prolongation of the template bleeding time and inhibition of ADP-induced platelet aggregation. G4120, a synthetic low-molecular-weight GPIIb/IIIa inhibitor that may be produced by chemical synthesis, may be of clinical value as a conjunctive agent for thrombolysis in patients with ischemic coronary syndromes. (Arteriosclerosis and Thrombosis 1993;13:738–747)

KEY WORDS • acute myocardial infarction • thrombolytic therapy • arterial reocclusion • template bleeding time • glycoprotein IIb/IIIa receptor

It is well established that thrombolytic therapy improves the outcome of acute myocardial infarction and that the conjunctive use of anticoagulants and/or antiplatelet agents increases the efficacy of coronary arterial thrombolysis. The efficacy of heparin and aspirin in enhancing and sustaining coronary arterial recanalization in humans is, however, limited. While more specific thrombin inhibitors and antiplatelet agents are more potent antithrombotic agents or conjuncts for thrombolytic therapy in experimental animal models. Recently we have reported that kistrin, a 68–amino acid snake venom polypeptide that inhibits the platelet glycoprotein (GP) IIb/IIIa receptor, increases the rate and extent of thrombolysis with a reduced dose of recombinant tissue-type plasminogen activator (rt-PA) and delays or abolishes reocclusion. Elucidation of the tertiary structure of this polypeptide has revealed that the Arg-Gly-Asp (RGD) adhesion site recognition sequence for the binding to the platelet GPIIb/IIIa receptor is located at the apex of a long loop across the surface of the polypeptide, which is fairly extended and has little contact with the rest of the molecule. The high affinity for GPIIb/IIIa of kistrin, as well as that of other RGD-containing proteins from snake venoms that lack structural homology outside the RGD region, was postulated to be due to favorable conformational re-
Animal Experiments

with thrombolytic agents in patients with acute myocardial
rivatives may have a 100-1,000-fold higher affinity.15-19
containing peptides have been shown to bind with low
comparable to that of kistrin, the former could constitute
occurrence of secondary binding determinants. 14 In
straints of the RGD recognition sequence and not to the
weight 668], were supplied by Genentech Inc., South
graphic catheter. Thoracotomy was performed through
anesthesia with pentobarbital (30 mg/kg body wt i.v. and
thetized with pentobarbital (30 mg/kg per minute i.v., were given for prophylaxis of
mg/kg per minute i.v., were given for prophylaxis of
methylglumine diatrizoate with videotape recording.

Methods

The single-chain rt-PA (Activase) and G4120 (L-
cysteine, N-(mercaptoacetyl) d-tyrosyl-l-arginylglycyl-
L-a-aspartyl-cyclic (1→5)-sulfide, 5-oxide [molecular
weight 668]), were supplied by Genentech Inc., South
SAN FRANCISCO, Calif. Heparin was purchased from
Elkins-Sinn, Cherry Hill, N.J.

Animal Experiments

The canine left anterior descending coronary arterial
thrombosis model with superimposed intimal damage
and high-grade stenosis was used exactly as previously
described.21 Adult mongrel dogs (20–25 kg) were anes-
thetized with pentobarbital (30 mg/kg body wt i.v. and
additional doses as required). The dogs were intubated
and placed on a respirator with a tidal volume of 10–15
mg/kg. Procainamide, 1.5 g i.m., and lidocaine, 0.1
mg/kg per minute i.v., were given for prophylaxis of
arrhythmias. The left carotid artery was exposed through an incision in the neck and cannulated with a
No. 7-1 modified Amplatz Judkins coronary angiog-
graphic 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
suspended to create a pericardial cradle. The left ante-
rior descending coronary artery was dissected, and a
2.5-cm segment was isolated distal to the first diagonal
branch. A 0.7-mm-i.d. catheter was inserted into a side
branch of the isolated left anterior descending coronary
artery segment, and an ultrasonic flow probe (T101,
Transsomic 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 using 1–2 mL
meglumine diatrizoate with videotape recording.

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 con-
stricted 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 base-
line. A control angiogram was then performed to visu-
alyze the coronary anatomy and baseline flow pattern
after flow reduction after placement of the stenosis.

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 carried out to damage the endothe-
lium and to promote thrombus adherence. One millili-
ter of blood was then drawn for thrombus formation.

Snare occlusions were made distal to the flow probe
and proximal to the constriction site. Thrombin (0.1 mL
of 100 units/mL, Thrombostat, Parke-Davis, Morris
Plains, N.J.) and 0.3 mL of blood were sequentially
jected through the side branch catheter into the emptied coronary artery segments, and the side branch
catheter was securely closed with a stopcock. After 5
minutes the proximal snare was released, and 2 minutes
later the distal tourniquet was released.

In the intravenous study of G4120, four treatment
groups were evaluated, a control group given intrave-
nous heparin alone and three groups with heparin and
an additional intravenous infusion of G4120 at a dose of
0.3, 0.2, or 0.1 mg/kg, given as a bolus of approximately
30% of the total dose followed by infusion of the
remainder over 60 minutes. Intravenous infusion of
G4120 was carried out with a constant-rate Harvard
infusion pump, starting after the confirmation of stable
complete coronary artery occlusion for 30 minutes by
Doppler flow probe and by coronary angiography. Ten
minutes later rt-PA was given as an intravenous bolus
injection of 0.45 mg/kg at 15-minute intervals until
recanalization of the thrombosed coronary artery was
achieved or until a maximum of 4 boluses was given.

In the study of endobronchial administration of
G4120, four groups were evaluated in a prospective,
randomized, blinded study design, a control group given
heparin alone and three groups with heparin and, in
addition, a single endobronchial instillation of G4120
at a dose of 0.5, 0.25, or 0.13 mg/kg.

G4120 was given as a solution of 2 mL, which was
delivered via a 4F angiographic catheter that was intro-
duced into the main bronchi through the endotracheal
tube. G4120 was administered after the confirmation of
stable complete coronary artery occlusion for 30 min-
utes by coronary angiography. Ten minutes later, rt-PA
was given as an intravenous bolus injection of 0.45
mg/kg at 15-minute intervals until recanalization of the
thrombosed coronary artery was achieved or until a
maximum of 4 boluses was given. Angiograms were
performed every 15 minutes to monitor occlusion and
additionally when the flow probe showed evidence of
reflow. Reflow was monitored both angiographically and
by flow probe for at least 2 hours after the initial
angiographic confirmation of stable coronary artery
occlusion. The animals were killed an average of 150
minutes after the start of the G4120 infusion.

The reflow time was defined as the time from the first
rt-PA bolus injection until recanalization was docu-
mented by the return of blood flow in the artery to
>25% of poststenotic flow as confirmed by complete
**Hemostasis and Pathological Analyses**

Bleeding times were performed before and at 30, 90, and 150 minutes after the initiation of G4120 administration with a spring-loaded blade device (Surgicutt International, Technidyne Corp., Edison, N.J.) applied to a shaved foreleg. Blood samples for assays of fibrinogen, t-PA antigen, platelet count, and platelet aggregation were obtained and processed as previously described.20 These studies involving experimental animals conformed to the Position of the American Heart Association on Research Animal Use, adopted November 11, 1984.

**Statistical Analysis**

The values are reported as mean±SD. The significance of differences between groups was determined with Student's t test for paired or unpaired values using the sst1 statistical package (BBN Software Products Corp., Cambridge, Mass.). The times to reperfusion were not normally distributed as determined by the Wilk-Shapiro test of the sst1 program. Therefore, for statistical analysis, these data were expressed as ln[(time)+1] values, which were normally distributed. Kruskal-Wallis22 exact tests were used to compare the occurrence of reflow and reocclusion and the number of rt-PA boluses in the various groups.

**TABLE 1. Effects of G4120 on Coronary Artery Recanalization and Reocclusion With rt-PA**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose of G4120 (mg/kg)</th>
<th>n</th>
<th>Blood flow (mL/min)</th>
<th>Coronary patency status (frequency)*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baseline</td>
<td>Poststenotic</td>
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<tr>
<td>Intravenous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>...</td>
<td>10</td>
<td>13±1.8</td>
<td>4.8±0.8</td>
</tr>
<tr>
<td>G4120</td>
<td>0.30</td>
<td>6</td>
<td>12±2.0</td>
<td>5.0±1.0</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>7</td>
<td>11±0.4</td>
<td>5.0±1.0</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>6</td>
<td>11±1.0</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>Endobronchial</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Heparin</td>
<td>...</td>
<td>5</td>
<td>13±1.2</td>
<td>4.6±0.7</td>
</tr>
<tr>
<td>G4120</td>
<td>0.50</td>
<td>5</td>
<td>13±2.0</td>
<td>6.2±1.2</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>6</td>
<td>13±3.2</td>
<td>5.2±1.3</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>5</td>
<td>11±1.2</td>
<td>4.5±0.9</td>
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</tbody>
</table>

rt-PA, recombinant tissue-type plasminogen activator; PO, persistent occlusion; CR, cyclic reocclusion and reflow after initial recanalization; PP, persistent patency after initial reflow. The data represent mean±SD. 

*p=0.008 for overall differences between groups and p=0.001 for the difference between control and 0.30 mg/kg G4120 groups in the intravenous study; p=0.008 for overall differences between groups and p=0.008 for the difference between control and 0.50 mg/kg G4120 groups in the endobronchial study (Kruskal-Wallis exact test).
reocclusion and reflow occurred in four of these seven dogs (Figure 1A). With 0.1 mg/kg G4120, reperfusion required more than one rt-PA bolus in two of six animals; reflow obtained in four animals was followed by cyclic reocclusion and reflow in two dogs.

Several significant differences in coronary arterial patency were observed with the various infusion protocols. Kruskal-Wallis exact tests yielded a probability value of 0.008 for overall differences between groups, \( p = 0.001 \) for controls versus 0.3 mg/kg G4120, and \( p = 0.03 \) for controls versus 0.2 mg/kg G4120. The time to reflow was significantly shorter in the group receiving 0.3 mg/kg G4120 than in the control group receiving heparin alone (\( p = 0.036 \) by \( t \) test performed on logarithmically transformed normalized data). The number of boluses of rt-PA plasminogen activator required to induce reflow was significantly lower in the 0.3 mg/kg G4120 group than in the control group (\( p = 0.03 \)).

In the study with randomized, blinded endobronchial instillation of G4120, bolus injection of rt-PA in control animals induced recanalization in four of five animals as illustrated in Figure 1B. Reflow in these animals was achieved within 52±72 minutes (mean±SD), using a value of 180 minutes for the animal with persistent occlusion. Cyclic reocclusion and reflow after initial reflow occurred in all four reperfused dogs (Figure 1B).

### Table 2. Effects of G4120 on Time to Reperfusion and Number of rt-PA Boluses

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose of G4120 (mg/kg)</th>
<th>No./total</th>
<th>Time (min)</th>
<th>ln[1+(time)]*</th>
<th>Median</th>
<th>Range</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>8/10</td>
<td>45±58</td>
<td>3.2±1.1</td>
<td>2</td>
<td>1-4</td>
</tr>
<tr>
<td>G4120</td>
<td>0.30</td>
<td>6/6</td>
<td>8±4</td>
<td>2.1+0.5</td>
<td>1</td>
<td>1-1</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>7/7</td>
<td>17±15</td>
<td>2.5±0.9</td>
<td>1</td>
<td>1-4</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>4/6</td>
<td>49±57</td>
<td>2.9±1.8</td>
<td>1</td>
<td>1-4</td>
</tr>
<tr>
<td>Endobronchial study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>...</td>
<td>4/5</td>
<td>52±72</td>
<td>3.4±1.2</td>
<td>2</td>
<td>1-4</td>
</tr>
<tr>
<td>G4120</td>
<td>0.50</td>
<td>5/5</td>
<td>7±5</td>
<td>1.9±0.6</td>
<td>1</td>
<td>1-1</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>6/6</td>
<td>14±17</td>
<td>2.4±0.8</td>
<td>1</td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>5/5</td>
<td>19±15</td>
<td>2.8±0.7</td>
<td>1</td>
<td>1-3</td>
</tr>
</tbody>
</table>

rt-PA, recombinant tissue-type plasminogen activator. The data are mean±SD; a value of 180 minutes was used for animals with persistent occlusion.

\( *p=0.036 \) for the difference between the control and 0.3 mg/kg G4120 groups in the intravenous study and \( p=0.039 \) for the difference between the control and 0.5 mg/kg G4120 groups in the endobronchial study.
The median number of rt-PA boluses required to induce reflow was 2, with a range of 1–4 (Table 2). Intravenous bolus injection of rt-PA in animals given 0.5 mg/kg endobronchial G4120 induced recanalization in all five animals (Figure 1B) within 7±5 minutes (Table 2). Reflow without subsequent reocclusion was achieved in all animals with a single bolus of rt-PA (Table 2). Bolus injections of rt-PA combined with 0.25 mg/kg endobronchial G4120 induced reflow in all six dogs within 14±17 minutes. Brief periods of reocclusion occurred in four of these six dogs. With 0.13 mg/kg endobronchial G4120, reperfusion required more than one rt-PA bolus in two of five animals, and reflow was followed by cyclic reocclusion and reflow in all animals. The median number of rt-PA boluses was 1, with a range of 1–3.

Again, significant differences in coronary arterial patency were observed with the various infusion protocols. A Kruskal-Wallis exact test analysis of all experiments yielded \( p = 0.0008 \) for overall differences between groups and \( p = 0.008 \) for the difference between the 0.5 mg/kg endobronchial G4120 group and the control group. The time to reflow was significantly shorter in the group receiving 0.5 mg/kg endobronchial G4120 than in the control group receiving heparin alone (\( p=0.039 \) by \( t \) test performed on logarithmically transformed normalized data). The number of boluses of rt-PA required to induce reflow was lower in the 0.5 mg/kg G4120 group than in the control group, but the difference was not significant (\( p=0.17 \)).

Hemostasis Analyses

Serial template bleeding times were measured in all animals, with results summarized in Figure 2. In the control group of the intravenous G4120 study, given heparin and rt-PA, the template bleeding time did not change significantly throughout the experiment (Figure 2A). Intravenous G4120 in combination with rt-PA resulted in a dose-related prolongation of the bleeding time: with 0.3 mg/kg, from a baseline value of 3–4 minutes before, to >30 minutes within 30 minutes after the start of the G4120 infusion. With a 0.10 mg/kg dose of G4120, the bleeding time was prolonged, from 4.8±1.3 minutes before to 21±10 minutes during infusion. A tendency toward normalization of the bleeding time was observed near the end of the observation period.

In control animals of the endobronchial G4120 study that were given heparin and rt-PA, the template bleeding time did not change significantly throughout the experiment (Figure 2B). Endobronchial G4120 in combination with rt-PA and heparin produced a dose-related prolongation of the bleeding time. With 0.50 mg/kg of G4120 the bleeding time was prolonged, from 2.6±0.8 minutes before to >30 minutes within 30 minutes after the endobronchial instillation, and the prolongation persisted throughout the duration of the experiment. With 0.25 mg/kg endobronchial G4120, the bleeding time was prolonged from 3.3±1.5 minutes before to 20±7.9 minutes during infusion and with 0.13 mg/kg endobronchial G4120, from 3.9±1.3 minutes before to 8.2±4.7 minutes during infusion. The bleeding time prolongation was, however, reversible with near-normalization toward the end of the experiment.

Figure 3 illustrates the results of platelet function tests. Platelet counts did not change markedly (data not shown). In the intravenous G4120 study, ADP-induced platelet aggregation was totally inhibited during the G4120 infusion, with partial recovery toward the end of the experiment in the 0.1 and 0.2 mg/kg G4120 groups. In the control group platelet aggregation remained essentially unaltered throughout the experiment.

In the endobronchial G4120 study, ADP-induced platelet aggregation was impaired to an extent proportional to the G4120 dose, with partial recovery toward the end of the experiment in the group receiving 0.13 mg/kg. In the control group platelet aggregation remained essentially unaltered throughout the experiment.

Results of plasma G4120 levels are summarized in Figure 4. Intravenous infusion of 0.3 mg/kg (125 \( \mu \)g/kg bolus followed by infusion of 3 \( \mu \)g/kg per minute for 60 minutes) produced a steady plasma level of approximately 450 ng/mL (Figure 4A). After the end of the infusion G4120 was cleared from plasma in a biphasic mode to reach approximately 20% of the steady-state levels within 2 hours. Similarly, 0.2 mg/kg (75 \( \mu \)g/kg bolus followed by 2 \( \mu \)g/kg per minute for 60 minutes).
produced a steady-state plasma level of approximately 350 ng/mL with a postinfusion decrease to 25% within 2 hours. A dose of 0.1 mg/kg (25 μg/kg bolus and an infusion of 1 μg/kg per minute for 60 minutes) yielded a plasma level of approximately 150 ng/mL and a postinfusion reduction similar to that observed at the higher doses.

Endobronchial instillation of G4120 produced dose-related plasma levels (Figure 4B). Steady-state levels were reached within 20 minutes and were maintained for 2–3 hours. Steady-state levels were approximately 250, 125, and 40 ng/mL with instillation of 0.5, 0.25, and 0.13 mg/kg, respectively.

Results of plasma rt-PA levels, fibrinogen, and hematocrit are summarized in Table 3. Plasma rt-PA levels increased to between 2.8 and 5.1 pg/mL measured 1 minute after bolus injection and decreased to between 0.4 and 0.6 pg/mL within 10 minutes. The fibrinogen level in the different groups of animals decreased moderately but remained above 35% of baseline in all groups. The changes in hematocrit value were not significant and did not differ between control and G4120 groups (all probability values >0.1).

Pathology

In the intravenous G4120 study, segments that were not recanalized primarily contained occlusive red blood cell clots (Table 4). Segments with reocclusion after initial reflow contained occlusive thrombi composed of mixed red blood cell and platelet-rich clots (Figure 5, top panel). Scanning electron microscopy of persistently patent arterial segments in dogs given intravenous or endobronchial G4120 revealed an irregular intimal surface, denuded of endothelium, that was covered with platelets and a few neutrophils and red blood cells (Figure 5, middle and lower panels).

Discussion

The role of coronary artery thrombosis in the pathogenesis of acute myocardial infarction is well established, and thrombolytic therapy has been shown to produce reflow of occluded coronary arteries, to salvage myocardium at risk, and to reduce mortality. Current thrombolytic strategies, however, suffer from suboptimal recanalization rates and times to reperfusion and unacceptably high frequencies of reocclusion after reflow. Although mechanical occlusion due to plaque rupture and edema or intramural hemorrhage may occasionally be responsible for resistance to thrombolysis, these limitations appear to be primarily the consequences of the nature and dynamics of the formation and dissolution of a coronary thrombus, of the variable composition of platelet-rich and erythrocyte-rich zones
TABLE 3. Effects of G4120 on Hemostasis Parameters

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose of G4120 (mg/kg)</th>
<th>rt-PA antigen level (μg/mL)</th>
<th>Fibrinogen level (g/L)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>1 Minute after rt-PA</td>
<td>10 Minutes after rt-PA</td>
<td>Baseline</td>
</tr>
<tr>
<td>Intravenous</td>
<td></td>
<td></td>
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<tr>
<td>Heparin</td>
<td>...</td>
<td>3.2±1.0</td>
<td>0.43±0.20</td>
<td>1.6±0.7</td>
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<td>G4120</td>
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<td>4.2±1.4</td>
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<td>0.20</td>
<td>3.8±1.6</td>
<td>0.66±0.50</td>
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<td>5.1±1.3</td>
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<tr>
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<td>3.4±0.6</td>
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<td>0.41±0.25</td>
<td>2.0±0.4</td>
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rt-PA, recombinant tissue-type plasminogen activator.

with different sensitivity to thrombolytic agents, of the procoagulant and platelet-activating properties associated with in vivo activation of the fibrinolytic system, and of the residual thrombogenicity of the recanalized coronary artery. Therefore, it is not surprising that monotherapy consisting of a short-term infusion of thrombolytic agents cannot achieve maximal and persistent coronary recanalization and that the conjunctive use of anticoagulants, antiplatelet agents, or both enhances and sustains coronary artery recanalization.

One interesting and promising approach to conjunctive platelet inhibition with thrombolytic therapy consists of in vivo inhibition of the platelet GPIIb/IIIa receptor, which constitutes the final common pathway for in vivo platelet aggregation by several in vivo agonists. After the initial demonstration that the murine anti-GPIIb/IIIa antibody 7E3 accelerates coronary thrombolysis and prevents reocclusion, similar effects were obtained with RGD-containing snake venom polypeptides including bitistatin, kistrin, and echistatin. Recent determination of the tertiary structure of kistrin has revealed that RGD-containing snake venoms exert their antiplatelet effect through the RGD recognition sequence, which is maintained in a restrained tertiary configuration, without contribution from the secondary binding sites elsewhere in these molecules. This observation opens the possibility for drug design via simulation of the restrained RGD conformation. Recently, synthetic cyclic RGD-containing peptide derivatives have indeed been shown to possess a 100-1,000-fold higher affinity for the platelet GPIIb/IIIa receptor than linear tetrapeptides. They bind with a high affinity (Kd in the nanomolar range) to both resting and ADP-activated human platelets and, after in vivo injection in experimental animals, reversibly inhibit ex vivo platelet aggregation. One of these synthetic peptides, G4120 (L-cysteine, N-(mercaptoacetyl)-L-tyrosyl-L-arginylglycyl-L-α-aspartyl-cyclic(1→5)-sulfide, 5-oxide), inhibits fibrinogen binding to platelets with a median inhibitory concentration (IC50) of 2 nM and inhibits ADP-induced platelet aggregation with an IC50 of 150 nM. In rabbits, G4120 is cleared from the plasma with a terminal half-life of 55 minutes and prevents in vivo platelet deposition on injured arterial surfaces induced with balloon dilatation. G4120 was also found to be absorbed for >90% of the administered dose via the bronchial epithelium (S. Bunting et al, unpublished observations).

To delineate the potential conjunctive effect of intravenous or endobronchial administration of G4120 on thrombolysis, we have used a canine model of coronary artery thrombosis with superimposed high-grade stenosis, which has anatomic similarities to the coronary artery occlusion in patients with acute myocardial infarction. We have previously used the same model to investigate the effects of the monoclonal anti-GPIIb/IIIa antibody 7E3 and of kistrin on coronary arterial thrombolysis induced with rt-PA. Intravenous infusion as well as endobronchial instillation of G4120 at a dose of up to 0.3 and 0.5 mg/kg, respectively, accelerated coronary arterial thrombolysis with a reduced dose of rt-PA and delayed reocclusion. Acceleration of the lysis of an erythrocyte-rich clot with platelet inhibitors, as also previously demonstrated with the anti-GPIIb/IIIa antibody and with kistrin, is thus confirmed with G4120. One possible explanation for this observation is that platelet inhibition may prevent accretion of platelets on the dissolving clot at the site of a high-grade stenosis, a phenomenon that would counteract clot lysis. Administration of G4120 also prolonged the template bleeding time from approximately 4 to ≥30 minutes and abolished ADP-induced ex vivo platelet aggregation. These effects, obtained with a relatively small dose of G4120, are in agreement with the known high affinity of cyclic RGD peptides for the platelet GPIIb/IIIa receptor compared with linear tetrapeptides such as Arg-Gly-Asp-Tyr.

Prolongation of the bleeding time has previously been found to correlate with bleeding in this dog model, and visible oozing from surgical wounds was observed in the present study. It should, however, be stressed that the experimental protocol included both anticoagulation with heparin and antiplatelet therapy with G4120 and that an interactive effect between these agents with respect to bleeding time prolongation cannot be excluded. Prolongation of the template bleeding time in patients with acute myocardial infarction who have been treated with alteplase correlates with a tendency toward spontaneous clinical bleeding. Therefore, the present observation that G4120 markedly prolongs the bleeding...
### Table 4. Pathological Analysis of Arterial Segments

<table>
<thead>
<tr>
<th>Dose of G4120 (mg/kg)</th>
<th>Dog No.</th>
<th>SEM/H&amp;E</th>
<th>Patency status</th>
<th>Description</th>
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<td>5</td>
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Endobronchial study

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<th>Dose of G4120 (mg/kg)</th>
<th>Dog No.</th>
<th>SEM/H&amp;E</th>
<th>Patency status</th>
<th>Description</th>
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<td>5</td>
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<td>1</td>
<td>PML, rare RBCs</td>
</tr>
</tbody>
</table>

SEM/H&E, analysis by scanning electron microscopy or by histological analysis with hematoxylin and eosin staining; PML, platelet monolayer; MPE, mixed thrombus with interlaced platelet-rich and erythrocyte-rich zones; RBCs, red blood cells; ER, erythrocyte-rich thrombus; PR, platelet-rich thrombus; WBCs, white blood cells.

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Time is a matter of concern. This concern is further highlighted by the observation that effective prevention of reocclusion after thrombolysis only occurs at doses of G4120 that markedly prolong the bleeding time and that the reocclusion process resumes with clearance of G4120 from plasma and with normalization of the bleeding time and ADP-induced platelet aggregation. Nevertheless, the potential iatrogenic bleeding tendency in humans as induced with G4120 might be partially offset by the use of a reduced dose of rt-PA required to induce clot lysis and by the rapid reversibility of the pharmacological effects of G4120.

Our results obtained with intravenous administration of G4120 are similar to our earlier results obtained with kistrin. On a weight basis, G4120 (molecular weight of 668) appears to be roughly equipotent to kistrin (molecular weight of 7,500) in terms of the acceleration of clot lysis, delay of reocclusion, prolongation of the bleeding time, and inhibition of ADP-induced platelet aggregation. Also, the clearance rates that are more precisely defined for G4120 than for kistrin appear to be not markedly different. Consequently, the major advantage of G4120 over kistrin, in the face of comparable pharmacodynamics and efficacy, would consist of the availability of the low-molecular-weight compound...
by chemical synthesis, whereas production of the high-
molecular-weight kistrin would require a recombinant
DNA approach.

The results of the present study are potentially sig-
ificant with respect to the use of GPIIb/IIIa inhibitors
for the prevention of coronary arterial occlusion or
reocclusion in acute coronary syndromes or after me-
chanical or thrombolytic recanalization, as well as for
the acceleration of coronary arterial thrombolysis. The
relatively short-acting effect of intravenously adminis-
tered G4120 renders it more adaptable to various
clinical situations. The observation that endobronchial
instillation of G4120 rapidly results in pharmacological
blood levels that produce complete and prolonged
inhibition of ADP-induced platelet aggregation suggests
that its administration by inhalation might be a viable
and valuable alternative to continuous intravenous in-
fusion for prehospital treatment. However, potential

FIGURE 5. Scanning electron photomicro-
graph (SEM) of left anterior descending
 coronary arterial segments. Upper panel:
From a control dog treated with heparin
 and recombinant tissue-type plasminogen
 activator, which resulted in recanalization
 with cyclic reflow and reocclusion. SEM
 reveals an intimal surface coated with plate-
lets and occasional red blood cells and red
blood cell-platelet clumps, x 1,000. Middle
panel: From a dog treated with 0.3 mg/kg
intravenous G4120. The surface is coated
by flattened platelets and a few neutrophils
and red blood cells. x 1,400. Lower panel:
From a dog treated with 0.5 mg/kg en-
dobronchial G4120. The surface is covered
with a layer of platelets that have undergone
metamorphosis. x 2,800.
effects of G4120 on the bronchial epithelium remain to be explored. Clearly, an orally active variant of G4120 would be preferable, but the development of a bioavailable peroral compound might require extensive drug design efforts.

It is hoped that further development in this field of research, which has evolved from the use of a murine monoclonal anti-GPIIb/IIIa antibody over snake venom polypeptides to low-molecular-weight synthetic peptide derivatives, will progress to the development of orally active compounds, with dissociation of their antithrombotic and hemorrhagic properties.

Acknowledgments

The authors thank Ms. Missy Stanton for excellent secretarial assistance, Ms. Kelly Fitzpatrick and Mr. Robert Holt for blood analysis, Ms. Tracy Swizzer for expert surgical assistance, Mr. John B. Newell, Cardiac Computer Center, Massachusetts General Hospital, for the statistical analyses, and the Peptide Group of the Biomedical Chemistry Department for the G4120 assays.

References

Intravenous and endobronchial administration of G4120, a cyclic Arg-Gly-Asp-containing platelet GPIIb/IIIa receptor-blocking pentapeptide, enhances and sustains coronary arterial thrombolysis with rt-PA in a canine preparation.

T Yasuda, H K Gold, C Kohmura, L Guerrero, H Yaoita, J T Fallon, S Bunting and D Collen

doi: 10.1161/01.ATV.13.5.738

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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