Cilostazol, a Novel Cyclic AMP Phosphodiesterase Inhibitor, Prevents Reocclusion After Coronary Arterial Thrombolysis With Recombinant Tissue-Type Plasminogen Activator

Shuichi Saitoh, Tomiyoshi Saito, Atsushi Otake, Takayuki Owada, Minoru Mitsugi, Hiromichi Hashimoto, and Yukio Maruyama

Inhibitors of cyclic nucleotide phosphodiesterase hydrolysis of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate are known to inhibit platelet aggregation, which plays an important role in acute reocclusion after thrombolysis in acute myocardial infarction. In the present study of a canine preparation of coronary artery thrombosis superimposed on high-grade stenosis, we tested whether the antithrombotic agent cilostazol, an inhibitor of cAMP phosphodiesterase, could prevent acute reocclusion or sustain coronary blood flow after thrombolysis when used with recombinant tissue-type plasminogen activator (rt-PA) and heparin. Intravenous infusion of rt-PA (0.5 mg/kg body wt for 30 minutes) and heparin (a 150 IU/kg body wt i.v. bolus and then 25 IU/kg body wt per hour i.v.) was combined with cilostazol (0.6 or 1.8 mg/kg body wt for 60 minutes). Without cilostazol, reperfusion was observed in seven of eight dogs, but reocclusion occurred in six of these seven dogs after 9±2 minutes. After administration of 1.8 mg/kg body wt cilostazol (group B-2; a 120-minute observation after the start of rt-PA infusion), reperfusion occurred in all seven dogs (p<0.05 versus control group), and brief cyclic reocclusion was observed in only one dog 63 minutes after reperfusion. At the same dose of cilostazol (group B-2L; a 240-minute observation after the start of rt-PA infusion), reperfusion occurred in all five dogs (p<0.05 versus control group), and coronary blood flow was well maintained except for one short reocclusion in one dog. Cilostazol inhibited cyclic flow reduction in a dose-dependent fashion. We conclude that cilostazol is a new potent antiplatelet agent for preventing reocclusion after coronary thrombolysis, when used in combination with rt-PA and heparin. (Arteriosclerosis and Thrombosis 1993;13:563-570)

KEY WORDS • acute myocardial infarction • recombinant tissue-type plasminogen activator • cilostazol • cyclic AMP phosphodiesterase inhibitor

It is generally accepted that platelet adhesion and aggregation play an important role in the pathogenesis of thrombosis, particularly arteriothrombosis.1-3 Coronary artery thrombolytic therapy in myocardial infarction, which is mainly accompanied by coronary arterial thrombosis, has advanced remarkably in recent years through the use of thrombolytic agents such as recombinant tissue-type plasminogen activator (rt-PA). However, continuous thrombosis triggered by platelet activation may result in reocclusion, which complicates 15-20% of cases.4-7 In particular, patients with high-grade (80% or greater) residual stenosis after thrombolysis are considered to be at the greatest risk for acute reocclusion.8 A number of antiplatelet drugs have been evaluated for their effects on reperfusion and reocclusion by thrombosis or its recurrence.9,10 Acetylsalicylic acid,11 dipyridamole,12 and prostacyclin analogues13 are representative drugs of this class, but their effects remain controversial.14-15 The unsatisfactory therapeutic effects of these antiplatelet drugs appear to be due to the insufficient potency of their pharmacological action, indicating that a new antithrombotic drug with higher effectiveness must be developed. A new synthetic platelet-aggregation inhibitor, cilostazol (6-[4-(1-cyclohexyl-L)tetrazol-5-yl)butoxy]-3,4-dihydro-2-(1H)-quinolinone), has a specific inhibitory action on cyclic AMP (cAMP) phosphodiesterase.16 In in vitro experiments, the drug caused a marked inhibition of aggregation of human platelets induced by ADP, collagen, arachidonic acid, epinephrine, thromboxane A2, and platelet activating factor.17 Thus, the aim of this study was to compare the effects on thrombolysis and reocclusion of rt-PA, either alone or in combination with cilostazol, in a heparinized canine model of coronary artery thrombosis in the presence of a flow-limiting critical stenosis.

Methods

Thrombolytic and Antiplatelet Agents

Single-chain rt-PA (GMK-527) was supplied from Kyowa Hakko Kogyo Co., Ltd., Tokyo. The phosphodi-
esterase inhibitor cilostazol was supplied from Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan. Cilostazol was dissolved in an N,N-dimethylformamide/saline solvent system for administration.

**Coronary Artery Thrombosis**

A previously described myocardial infarction model was used. Male or female purposely bred mongrel dogs (11–28 kg) were anesthetized with sodium pentobarbital (30 mg/kg body wt i.v. and additional doses as required) and ventilated with room air by a positive-pressure ventilator after intubation. Lidocaine (2 mg/kg body wt bolus i.v.) was given as prophylactic antiarrhythmic therapy. The left subcutaneous lower-limb vein was cannulated for the maintenance infusion of saline and the infusion of rt-PA and heparin. The right carotid artery was cannulated for the measurement of mean arterial blood pressure. A thoracotomy was performed in the fifth left intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated 1.5 cm distal to the first diagonal branch, and side branches were ligated. An electromagnetic flow probe (Nihon Koden, MFV-2100) was placed in the proximal portion of the LAD for measurement of coronary blood flow. One milliliter of blood was drawn for thrombus formation. After the administration of intravenous heparin (bolus injection of 150 IU/kg body wt and then 25 IU/kg body wt at hourly intervals), a 2-mm-wide plastic wire tie was progressively constricted around the LAD, just distal to the proposed site of thrombus formation, to reduce the blood flow to 40±10% of the baseline rate. After the left carotid artery cutdown, a modified right Judkins catheter was advanced under fluoroscopic control to the left main coronary artery. Fluoroscopic images were obtained with a Toshiba x-ray imaging system. An angiogram of the LAD with videotape recording was obtained with a 1–2-mL iopamidol injection. A control angiogram was then obtained to confirm the presence of more than 90% stenosis. The 1 cm of coronary artery proximal to the wire tie was isolated with just proximal and distal temporary silk snares. Endothelial damage was induced by grasping the segment with blunt forceps, thereby promoting thrombus adherence. Thrombin (0.01 mL; 1,000 units/mL solution) mixed with 0.3 mL blood was injected through the side-branch catheter into the collapsed coronary artery segment that had been isolated previously. After 15 minutes, the snares were released. An angiogram was obtained 30 minutes after thrombus formation to observe angiographically the characteristics of the occlusion site and to confirm total coronary occlusion, although the latter was clearly shown by zero flow as measured with the electromagnetic flowmeter. If reflow occurred, another angiogram was immediately obtained, and coronary stenosis was angiographically characterized. If this was unsuccessful, angiograms were obtained at 15-minute intervals throughout the experiment. The left external jugular vein was cannulated for the continuous infusion of cilostazol. All experiments with animals were performed according to the guidelines of Fuskushima Medical College.

**Hemodynamic Measurements**

In addition to continuous coronary blood flow measurement, aortic blood pressure was monitored (AP641G, Nihon Koden) by inserting a catheter into the right carotid artery. Subdermal needle electrodes were inserted for the recording of a lead II electrocardiogram.

**Administration of rt-PA and Cilostazol**

After confirmation of a totally occlusive thrombus as mentioned above, two treatments for coronary reperfusion and then reocclusion were tested: 1) rt-PA and heparin (group A, n=8); 2) rt-PA, heparin, and the combination of two different doses of cilostazol (0.6 mg/kg body wt for 60 minutes in group B-1 [n=5] and 1.8 mg/kg body wt for 60 minutes in group B-2 [n=7] and group B-2L [n=5]). rt-PA was infused intravenously at 17 μg/kg body wt per minute for 30 minutes. At the beginning of rt-PA infusion, 20% of the total dose was given as a bolus. Heparin was injected at 150 IU/kg body wt intravenously before rt-PA infusion and was followed by 25 IU/kg body wt at hourly intervals. rt-PA and cilostazol infusions were started simultaneously via different infusion lines.

Time to reperfusion was taken as the time from the beginning of rt-PA infusion until reperfusion was established, which 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 three heart beats or fewer. After recanalization was achieved, blood flow was monitored for evidence of recollection, defined as blood flow less than 25% of baseline flow. The recollection time was the interval between the documented first reperfusion and first recollection.

Experimental observation time in group A, group B-1, and group B-2 was 120 minutes after the beginning of rt-PA infusion. In group B-2L, the observation period was 240 minutes.

**Hemostasis Analysis**

Platelet aggregation rate was measured before infusion and at 30, 120, and 240 minutes (group B-2L only) after infusion of rt-PA. Venous blood samples taken from the right ventricle for platelet aggregation tests were collected in 0.01 M citrate and 200 kallikrein inhibitor units (KIU) aprotinin per milliliter (Sigma Chemical Co., St. Louis, Mo.). Platelet aggregation in whole blood was determined by impedance aggregometry with a whole-blood platelet aggregometer, using a method modified from Cardinal and Flower. The stimulus for whole-blood platelet aggregation was collagen (5 mg/mL). Platelet-rich plasma was prepared, and platelet aggregation induced with ADP was tested by using a method modified from Born and Cross. Venous blood samples collected in citrate and kallikrein-containing solution were also obtained for platelet counts.

Bleeding time after cutting of a shaved portion of one foreleg with a spring-loaded blade device (Surgicutt International, Technidyne Corp., Edison, N.J.) was measured before infusion and at 30, 120, and 240 minutes (group B-2L only) after infusion of rt-PA.
The plasma concentration of cilostazol (groups B-1, B-2, and B-2L) was measured at 30, 120, and 240 minutes (group B-2L only) after cilostazol infusion. Two milliliters of blood taken from the right ventricle was added to 3.13% citrate, immediately placed on ice, and then centrifuged at 1,500g for 10 minutes; plasma samples were stored at −70°C for measurement of cilostazol plasma concentration, which was determined within 4 weeks by the method of Akiyama et al.21

**Pathological Examination**

At the end of the experiment, the LAD was perfusion fixed in situ with 5% formaldehyde solution. The heart was then removed and immersion fixed in 5% formaldehyde. The thrombosed and stenotic segments were dissected and cross sectioned, processed for light microscopy, and stained with hematoxylin and eosin.

**Statistical Analysis**

All data are expressed as the mean±SEM. Hemodynamic parameters were analyzed by one-way analysis of variance, followed by unpaired Student’s t test. Fisher’s exact test was used to determine significant differences in the incidence of reocclusion between control and cilostazol treatments. Hemostatic parameters were analyzed by a Kruskal-Wallis nonparametric analysis of variance, followed by the Wilcoxon-Mann-Whitney test. This form of analysis of variance was selected because of the nongaussian distribution of the hemostatic variables. A probability value less than 0.05 was considered significant.

**Results**

**Comparative Thrombolytic Effects of rt-PA and Cilostazol Plus rt-PA in the Heparinized Model**

The comparative thrombolytic effects among groups A, B-1, B-2, and B-2L are summarized in Table 1. The patency status in the individual animals is also schematically represented in Figure 1. Drug infusion time (approximately 15 minutes) from the formation of the occlusive thrombus was similar in each group. rt-PA with heparin (group A) caused reperfusion in seven of eight dogs, showing a mean time to reperfusion of 20±5 minutes (range, 3–39 minutes). Cyclic reflow and reocclusion occurred in six of seven dogs after 9±2 minutes (range, 5–11 minutes). Infusion of rt-PA and 0.6 mg/kg body wt cilostazol (group B-1) induced reperfusion (time to reperfusion, 7±2 minutes; range, 2–11 minutes) in all five dogs. Again, reocclusion was observed in four of five dogs after 38±18 minutes. Infusion of rt-PA and 1.8 mg/kg body wt cilostazol (group B-2) induced reperfusion in all seven dogs with a mean time to reperfusion of 14±4 minutes (range, 2–30 minutes). In only one dog was brief reocclusion observed (duration of reocclusion was 1 minute) after 63 minutes. In the same dog, two 1-minute reocclusions occurred after the second reperfusion. Reocclusion did not occur in any other of the six of seven dogs in group B-2 (p<0.05 vs. group A). In group B-2L, reperfusion (time to reperfusion, 9±2 minutes; range, 4–20 minutes) occurred in all five dogs, and in one of five dogs, reocclusion was observed after 13 minutes and reperfusion occurred after 4 minutes. The other four dogs in group B-2L did not show reocclusion, and coronary blood flow was satisfactorily sustained (p<0.05 vs. group A). Thus, also in a longer observation time, the efficacy of treatment with rt-PA and 1.8 mg/kg body wt cilostazol was still maintained.

**Hemodynamic Data**

Mean blood pressure measurements are summarized in Table 2. During cilostazol infusion, mean blood pressures decreased in a dose-dependent fashion. Heart rates did not differ among the groups at any time point tested.

**Hemostasis Analysis**

Bleeding times measured in all animals are summarized in Table 3. In control dogs (group A), the template bleeding time did not change significantly throughout the experiment. Cilostazol in combination with rt-PA tended to prolong bleeding time in a dose-dependent fashion. In one of eight dogs in group A, we could not obtain an exact measurement because of technical problems, and therefore, the data for this dog were not used.

Platelet counts did not change significantly in any group (group A, 221±27.2; group B-1, 251±33.8; group B-2, 228±26.7; and group B-2L, 231±29.8 [×103/μL for all values]).

Table 4 summarizes the results of ADP-induced platelet-aggregation tests. Cilostazol administration in combination with rt-PA appeared to show a dose-related depression of ADP-induced platelet aggregation at 120 minutes after cilostazol infusion (Table 4); with 1.8 mg/kg body wt cilostazol, platelet aggregation was depressed to 30±7% in group B-2 (six dogs; p<0.05 vs.
FIGURE 1. Schematic representation of the left anterior descending coronary arterial patency status in dogs receiving a continuous infusion of 0.5 mg/kg recombinant tissue-type plasminogen activator (rt-PA), heparin, and cilostazol. H, Heparin injection; a, continuous intravenous infusion of rt-PA; m, continuous intravenous infusion of cilostazol. Min., minutes.

Table 2. Mean Blood Pressure Responses During Coronary Artery Thrombolysis

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8</td>
<td>103±6</td>
<td>100±8</td>
<td>98±7</td>
<td>96±5</td>
<td>94±6</td>
<td>95±4</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B-1</td>
<td>5</td>
<td>101±8</td>
<td>97±8</td>
<td>87±7</td>
<td>81±4</td>
<td>79±3</td>
<td>75±3</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B-2</td>
<td>7</td>
<td>100±7</td>
<td>86±6</td>
<td>82±4</td>
<td>77±4</td>
<td>74±4</td>
<td>70±5*</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>B-2L</td>
<td>5</td>
<td>105±7</td>
<td>88±7</td>
<td>78±6</td>
<td>74±7</td>
<td>70±8*</td>
<td>68±6*</td>
<td>64±6</td>
<td>61±5</td>
</tr>
</tbody>
</table>

rt-PA, recombinant tissue-type plasminogen activator. The data are mean±SEM, and are in millimeters of mercury. See the footnote to Table 1 for an explanation of groups A, B-1, B-2, and B-2L.

*p<0.05 vs. group A.

Discussion

Coronary arterial thrombolytic therapy in acute myocardial infarction has become the standard of care for appropriately eligible patients in the past 3 years. Despite this major advance, recent large trials of thrombolytic therapy have shown a persistently high 30-day mortality rate of about 10%, irrespective of which thrombolytic agent, such as rt-PA, is administered. Moreover, significant residual left ventricular dysfunc-
tion despite thrombolytic therapy suggests that additional therapeutic strategies are needed.\textsuperscript{25} In addition, reocclusion after thrombolysis has also been reported to occur in approximately 15–25% of patients,\textsuperscript{28,29} and this induced myocardial damage as well as accidental bleeding is an inevitable problem with present-day thrombolytic therapy. In particular, this complication occurs frequently in cases of severe stenosis, thus causing further myocardial damage.\textsuperscript{29} Thus, to further improve the management of acute myocardial infarction, treatment of the coronary thrombosis and protection against reocclusion must be performed simultaneously.

Many antiplatelet and antiplatelet therapies have been tried for protection against reocclusion after thrombolysis in the clinical setting.\textsuperscript{27,30,31} However, it is quite difficult to assess the efficacy of these drugs in protecting against reocclusion, because the degree of coronary stenosis, extension or number of stenosed or occluded arteries, hemodynamics, platelet aggregation, and coagulability, which all influence coronary thrombosis, are different in each case. To overcome these difficulties, a coronary thrombosis model with high-grade stenosis developed in dogs by Gold and colleagues was used in the present study. However, it should be noted that this experimental coronary arterial thrombolytic model suffers some limitations when one attempts to extrapolate the beneficial results reported here to the clinical situation. First, this model requires large amounts of study material and requires open-chest procedures. Second, this canine model uses a mechanically damaged coronary artery rather than an atherosclerotic coronary artery, and adjunctive treatments were initiated after 30-minute periods of maturation rather than the 3–6-hour delay that occurs clinically. However, this canine model with superimposed endothelial damage and fixed high-grade stenosis simulates the anatomic features occurring in patients with acute myocardial infarction. Damage to the endothelium that exposes subendothelial thrombogenic structure, a uniform production of fixed high-grade stenosis (more than 90%), and the absence of spontaneous reflow constitute a reproducible quantitative model for the investigation of both coronary thrombolysis and approaches to prevent reocclusion. Pathological examination of the material responsible for reocclusion in this model revealed that it consisted of a platelet-rich thrombus, possibly of similar composition to the thrombolysis-insensitive material responsible for reocclusion in humans.\textsuperscript{33}

Using the present experimental model, we have investigated the effect of an aspirin and prostacyclin analogue on protection against reocclusion after coronary thrombolysis. Simultaneous or individual use of heparin as an antiplatelet and aspirin as an antiplatelet drug did not overcome this situation.\textsuperscript{13} This conclusion was reconfirmed by the present experiment (Figure 1 and Tables 1–5) and in the presence of a large amount of rt-PA.\textsuperscript{34} Other antiplatelet agents such as bitistatin, iloprost,\textsuperscript{15} or lipoprotein-associated coagulation inhibitors\textsuperscript{34} have been tested in the treatment of coronary reperfusion and reocclusion after thrombolysis or the recurrence of reperfusion and reocclusion in different experimental models. However, their efficacy is insufficient, and no ideal drug for this purpose has been reported. The prostacyclin analogue, for example, greatly decreased systemic mean blood pressure from 93±4 to 58±5 mm Hg,\textsuperscript{13} which therefore makes it difficult to use in the clinical setting.

Cilostazol, which was developed in 1984, inhibits human platelet aggregation induced by ADP, collagen, and arachidonic acids through selective platelet cAMP

### Table 3. Bleeding Time Measurements

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before rt-PA</th>
<th>End of rt-PA</th>
<th>2 Hours from rt-PA</th>
<th>4 Hours from rt-PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>4.5±0.7</td>
<td>6.3±0.8</td>
<td>4.5±0.9</td>
<td>...</td>
</tr>
<tr>
<td>B-1</td>
<td>5</td>
<td>3.7±0.2</td>
<td>8.4±1.4</td>
<td>5.9±1.3</td>
<td>...</td>
</tr>
<tr>
<td>B-2</td>
<td>7</td>
<td>4.1±0.6</td>
<td>10.3±1.9</td>
<td>6.6±1.4</td>
<td>...</td>
</tr>
<tr>
<td>B-2L</td>
<td>5</td>
<td>3.8±0.6</td>
<td>14.2±1.6*</td>
<td>6.5±0.8</td>
<td>6.1±1.2</td>
</tr>
</tbody>
</table>

rt-PA, recombinant tissue-type plasminogen activator. The data are mean±SEM. See the footnote to Table 1 for an explanation of groups A, B-1, B-2, and B-2L.

*p<0.05 vs. group A.

### Table 4. ADP-Induced Platelet-Rich Plasma Aggregation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>End of rt-PA</th>
<th>2 Hours from start</th>
<th>4 Hours from start</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>89±7</td>
<td>94±26</td>
<td>...</td>
</tr>
<tr>
<td>B-1</td>
<td>4</td>
<td>34±4</td>
<td>77±2</td>
<td>...</td>
</tr>
<tr>
<td>B-2</td>
<td>6</td>
<td>59±8</td>
<td>30±7*</td>
<td>...</td>
</tr>
<tr>
<td>B-2L</td>
<td>4</td>
<td>52±9</td>
<td>28±4*</td>
<td>39±14</td>
</tr>
</tbody>
</table>

rt-PA, recombinant tissue-type plasminogen activator. Results represent percentages of changes in light transmission compared with a baseline study performed before rt-PA infusion, i.e., the state without drug administration, in each corresponding dog. See the footnote to Table 1 for an explanation of groups A, B-1, B-2, and B-2L.

*p<0.05 vs. group A.

### Table 5. Collagen-Induced Platelet Aggregation

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>End of rt-PA</th>
<th>2 Hours from start</th>
<th>4 Hours from start</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>102±22</td>
<td>102±23*</td>
<td>...</td>
</tr>
<tr>
<td>B-1</td>
<td>4</td>
<td>56±9</td>
<td>70±11</td>
<td>...</td>
</tr>
<tr>
<td>B-2</td>
<td>6</td>
<td>49±14</td>
<td>77±25</td>
<td>...</td>
</tr>
<tr>
<td>B-2L</td>
<td>4</td>
<td>39±12</td>
<td>70±26</td>
<td>82±24</td>
</tr>
</tbody>
</table>

rt-PA, recombinant tissue-type plasminogen activator. Results represent percentages of changes in impedance compared with a baseline study performed before rt-PA infusion, i.e., the state without drug administration, in each corresponding dog. See the footnote to Table 1 for an explanation of groups A, B-1, B-2, and B-2L.

*pTwo-hour values for six of seven dogs only.
TABLE 6. Plasma Concentration of Cilostazol

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>End of rt-PA</th>
<th>2 Hours from start</th>
<th>4 Hours from start</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-1</td>
<td>5</td>
<td>238±34.4</td>
<td>105±28.4</td>
<td>⋮</td>
</tr>
<tr>
<td>B-2</td>
<td>6</td>
<td>589±32.1</td>
<td>488±42.1*</td>
<td>⋮</td>
</tr>
<tr>
<td>B-2L</td>
<td>5</td>
<td>536±44.8</td>
<td>429±52.6</td>
<td>339±49.6</td>
</tr>
</tbody>
</table>

*Two-hour values for five of six dogs only.

rt-PA, recombinant tissue-type plasminogen activator. See the footnote to Table 1 for an explanation of groups B-1, B-2, and B-2L.

phosphodiesterase inhibition, and also has a vasodilating action. In patients with peripheral arterial occlusion, cilostazol improved clinical signs and increased skin blood flow through its vasodilating and/or antiplatelet actions. Despite this remarkable effectiveness, this agent has undergone little investigation for acute reocclusion after coronary thrombolysis in myocardial infarction. The present study shows that cilostazol prevented reocclusion in a dose-dependent fashion, with a trend toward shortening the time to reperfusion compared with control when rt-PA and heparin are used simultaneously. That is, in group B-2 (combination of 1.8 mg/kg body wt cilostazol, rt-PA, and heparin), only one of seven reperfused dogs exhibited three brief cyclic reocclusions, and in group B-2L, only one of five reperfused dogs showed one brief reocclusion before 240 minutes after the start of rt-PA infusion (Figure 1). In contrast, in group B-1 (combination of 0.6 mg/kg body wt cilostazol, rt-PA, and heparin), reocclusion occurred in four of five dogs within 38±18 minutes. Possible reasons why reocclusion was almost completely prevented in the B-2 and B-2L groups are as follows: 1) Since cilostazol tended to suppress platelet aggregation induced by ADP and collagen in vitro (Tables 4 and 5), the number of platelets and other binding proteins that bind at the injured coronary arterial site by fibrinogen may be decreased. 2) Antiplatelet effects of cilostazol increased concomitantly with endogenous prostacyclin in vivo.39 3) Cilostazol may have some yet-unknown effectiveness in the prevention of reocclusion.

Yasuda et al,12 using the same model as ours, have reported the effects of another phosphodiesterase inhibitor, dipyridamole (0.6 mg/kg body wt i.v. bolus injection; maximum dose without hemodynamic compromise), on the prevention of reocclusion after thrombolysis with two-chain rt-PA (15 µg/kg body wt per minute for 30 minutes) and heparin. In their study, coronary reocclusion was found in five of six dogs within 11 minutes, indicating only minimal benefit in preventing reocclusion. This result may be related to the facts that 1) dipyridamole is an inhibitor of c guanosine monophosphate-1 and, therefore, has little effect on cAMP inhibition, and 2) in a clinical setting at the dose of dipyridamole used, the antiplatelet effect is mainly due to endogenous prostacyclin from the endothelium, which is produced by dipyridamole. Therefore, dipyridamole has hardly any effect on the suppression of platelet aggregation in itself.

FIGURE 2. Photomicrograph of fibrin and platelet-rich thrombus in the stenotic segment in one example from group B-2 (dog 2 of group B-2 shown in Figure 1). Hematoxylin and eosin stain, x40.
The plasma concentration of cilostazol found in the B-2 and B-2L groups at the end of rt-PA infusion was 589±321 ng/mL and 536±44.8 ng/mL, respectively; in the B-2L group, the value at 240 minutes from rt-PA infusion was 339±49.6 ng/mL. These values correspond well with those observed clinically after oral administration of the drug to humans (100 mg b.i.d.). This dose is usually considered either to have little hemodynamic effect clinically or to slightly decrease systemic blood pressure (approximately 5% 1 hour after oral administration). In the present study, cilostazol decreased blood pressure in a dose-dependent fashion, whereas Kawamura et al observed no pressure decrease after the same dose of cilostazol infusion in a closed-chest dog. One reason for the decreased systemic blood pressure in the present study may relate to our experimental condition of open-chest surgery. In this model, from the point of view of decreasing blood pressure (in the group B-2L mean blood pressure was decreased from 105±7 to 61±5 mm Hg at 240 minutes from rt-PA infusion), the longest possible duration of observation may be about 240 minutes after the infusion of cilostazol and rt-PA. Despite this blood pressure fall, satisfactory coronary blood flow was maintained. Thus, the present efficacy for preventing reocclusion can be expected in the conscious closed-chest condition without being influenced by serious hemodynamic disturbances.

Finally, the present study indicates that cilostazol, a cAMP phosphodiesterase inhibitor, can eliminate reocclusion very well after thrombolysis with rt-PA and heparin in acute coronary occlusion. Therefore, it seems likely that this combination therapy can be recommended to sustain recanalization duration after thrombolysis in patients with acute myocardial infarction. However, it should be noted that this model is not the same as the coronary artery thrombosis observed in humans and, again, these results were obtained in an open-chest anesthetized state. Thus, application of the present data must be made with care.

References


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doi: 10.1161/01.ATV.13.4.563

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