Antiplatelet Antibody [7E3 F(ab')2] Prevents Rethrombosis After Recombinant Tissue-Type Plasminogen Activator–Induced Coronary Artery Thrombolysis in a Canine Model

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Coronary artery rethrombosis can complicate initially effective thrombolytic therapy. Platelets interacting with injured vascular endothelium in a region along the coronary artery with reduced luminal cross-sectional area contribute to rethrombosis. The purpose of this study was to investigate the potential of the F(ab')2 fragment of the murine monoclonal antibody 7E3 [7E3 F(ab')2] to prevent rethrombosis after intracoronary clot lysis with recombinant tissue-type plasminogen activator (rt-PA) in an experimental model. The 7E3 F(ab')2 binds to the platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa), thereby preventing platelet-fibrinogen interaction and intravascular thrombus formation. Experimental coronary artery thrombosis was produced in the anesthetized dog by application of direct anodal current to the intimal surface of the left circumflex coronary artery in the region of an external stenosis. Lysis of the established intracoronary thrombus was achieved with the intravenous administration of rt-PA (25 mg) after which the animals were randomized into two groups. Group 1 (n=10) served as the control, receiving the saline diluent, and group 2 (n=9) received 7E3 F(ab')2, given as a single intravenous injection (0.8 mg/kg). The times required for occlusive thrombus formation, rt-PA–induced thrombolysis, and rethrombosis (if it occurred) were similar in the animals treated with saline and those treated with 7E3 F(ab')2. The initial left circumflex coronary artery blood flow was similar in both groups but decreased to a negligible level in group 1. In group 2, left circumflex coronary artery blood flow declined modestly (24±2 to 10±2 ml/min). Rethrombosis occurred in all animals in group 1 but in only two of nine animals in group 2 (p<0.05). Oscillations in coronary blood flow preceded rethrombosis in group 1, whereas 7E3 F(ab')2 stabilized left circumflex coronary artery blood flow patterns during the course of the experimental protocol (5.2±0.9 vs. 0.7±0.4 oscillations, respectively; p<0.05). Thrombus mass recovered from the left circumflex coronary artery at the conclusion of each experiment was greater in group 1 as compared with group 2 (7.0±2.3 vs. 1.5±0.7 mg, respectively; p<0.05). The area of left ventricle at risk for infarction was similar in both groups but infarct size, infarction/at risk assessed histochemically, was larger in group 1 than group 2 (35±9% vs. 6±4%, respectively; p<0.05). Platelet aggregation induced by ADP and arachidonic acid was similar at baseline for all of the animals. In each case, ADP-induced aggregation was diminished by 50% after the rt-PA infusion, and this correlated with the decrease in plasma fibrinogen concentration (p<0.05). Three hours after the single injection of 7E3 F(ab')2, platelet aggregation induced by either ADP or arachidonic acid was inhibited by 90% or more (p<0.05). In conclusion, antibody to platelet glycoprotein IIb/IIIa receptors [7E3 F(ab')2] prevented reocclusion of the recanalized vessel, stabilized coronary artery blood flow in the recanalized vessel, and was accompanied by an overall reduction in infarct size. Despite the fact that inhibition of the GPIIb/IIIa antibody inhibited platelet-fibrinogen interactions and rethrombosis, restoration of reperfusion coronary artery blood flow at prethrombosis levels was not maintained. The latter observation suggests that components in addition to platelets and small thrombi can affect coronary vasomotor tone in the damaged artery after rt-PA–induced thrombolysis. (Circulation 1990;81:617–627)
Early treatment of an evolving myocardial infarction with thrombolytic therapy, such as streptokinase or recombinant tissue-type plasminogen activator (rt-PA), has become accepted as a means of limiting myocardial injury associated with an acute ischemic event, which in most instances is related to thrombosis in the presence of atherosclerotic coronary artery disease.1–4 Despite the success of thrombolysis in initiating coronary artery reperfusion in 50–80% of patients, reocclusion of the recanalized artery occurs. With the use of adjunctive therapy (heparin, aspirin, dipyridamole, or any two or all three of these agents), the incidence of rethrombosis is reduced to about 20–30%, regardless of whether streptokinase or rt-PA is used as the initial thrombolytic agent.5,6 No adjunctive therapy, however, has been shown to eliminate rethrombosis.

A major factor in early coronary artery rethrombosis appears to be the interaction of platelets with the endothelial surface in the diseased arterial segment.7 Platelet surface receptors play an important role in both adhesion and aggregation. Platelet adhesion to endothelial surfaces is the initial step in hemostasis but platelet-endothelium adhesion alone produces a minimal threat of thrombotic occlusion.8 Platelet aggregation, however, leads to formation of a platelet-rich fibrin-containing thrombus, which could progress to occlude the vessel. The agonists that are thought to initiate platelet aggregation in vivo (i.e., ADP, epinephrine, collagen, and thrombin) are dependent on the binding of fibrinogen to the glycoprotein IIb/IIIa (GPIIb/IIIa) receptor.9 Development of monoclonal antibodies directed against the GPIIb/IIIa complex facilitates defining the mechanism of early coronary artery rethrombosis.10

There have been several studies in a canine preparation with localized left anterior descending coronary artery thrombosis where the F(ab′)2 fragment of the murine monoclonal antiplatelet antibody 7E3 [7E3 F(ab′)2] that binds to the platelet GPIIb/IIIa complex was administered 10 minutes before the rt-PA to determine initial thrombolytic efficacy and occurrence of rethrombosis.11,12 In the earlier studies, occlusive thrombi were produced by thrombin instillation into a segment of the coronary artery that had been subjected to external trauma and proximal to an angiographically determined critical stenosis of greater than 90% (external constrictor reducing blood flow to 40±10% of baseline). In the present study, we explored the efficacy of an intravenous injection of 0.8 mg/kg of the 7E3 F(ab′)2, in preventing rethrombosis after rt-PA–induced lysis of an intracoronary thrombus. We used an experimental model in which thrombosis was induced by electrical stimulation applied to the intimal surface of the left circumflex coronary artery (LCCA) at the point of an external stenosis.13 We found that administration of the 7E3 F(ab′)2 after rt-PA–induced thrombolysis not only prevented thrombotic coronary artery reocclusion but also stabilized coronary artery blood flow in the recanalized vessel and was associated with an overall reduction in infarct size. Despite inhibition of platelet-fibrinogen interactions and rethrombosis, restoration of coronary artery blood flow at a level similar to baseline was not maintained. This finding suggests that in addition to platelets and small intraluminal thrombi other factors can affect coronary vasomotor tone in the damaged artery after rt-PA–induced thrombolysis.

Methods

Reagents

Single-chain rt-PA was purchased from Genentech Inc., South San Francisco, California. The F(ab′)2 fragment of the murine monoclonal antiplatelet antibody 7E3 [7E3 F(ab′)2] was supplied by Centocor Inc. (Malvern, Pennsylvania). Super Immerse Blue dye was obtained from CIBA-Geigy, Glen Falls, New York. Thromboplastin C, calcium chloride (0.02 M), activated cephaloplatin reagent, thrombin reagent, and Owren’s veronal buffer were ordered from Becton, Dickinson and Co., Rutherford, New Jersey. Ci-Trol Coagulation Control, Latex Anti-Fibrinogen, and Sorensens’s glycine buffer were purchased from American Dade (Aguada, Puerto Rico). Sample tubes for fibrinogen degradation products (FDP) assays were obtained from Wellcome Laboratories, Beckham, England. Epinephrine hydrochloride was purchased from Calbiochem, Los Angeles, California. Triphenyltetrazolium chloride, sodium citrate, ADP, arachidonic acid, and any reagent used in the laboratory but not specifically mentioned were purchased from Sigma Chemical Co., St. Louis, Missouri.

Surgical Preparation and Instrumentation

Male mongrel dogs weighing 14–20 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.), endotracheally intubated, and ventilated with room air and positive pressure at a stroke volume of 30 ml/kg and a frequency of 12 breaths/min (Harvard Apparatus, South Natick, Massachusetts). Cannulas were placed into the left carotid artery for monitoring arterial blood pressure (Statham P23 pressure transducer, Gould Inc., Oxnard, California) and into the jugular vein for administering intravenous fluids or obtaining blood samples. The heart was exposed by a left thoracotomy through the fifth intercostal space. The left atrium was cannulated with polyeth-
ethylene tubing. A 2–3-cm segment of the LCCA was isolated from surrounding tissue by blunt dissection. The artery was instrumented from proximal to distal with an electromagnetic flow probe (Carolina Medical Electronics, Inc., King, North Carolina), stimulation electrode, and screw occluder. The stimulation electrode was constructed from a 25-gauge hypodermic needle tip attached to a 30-gauge Teflon-insulated silver-coated copper wire. The mechanical occluder was constructed of stainless steel in a “C” shape with a Teflon screw (2-mm diameter), which could be manipulated to control vessel circumference. The occluder was adjusted to decrease by 50% the hyperemic response of coronary blood flow to a 10-second occlusion without affecting baseline coronary blood flow. Continuous recordings of blood pressure, limb lead II electrocardiogram, and mean and phasic LCCA blood flow (CBF) were obtained on a model 7 polygraph recorder (Grass Instrument Co., Quincy, Massachusetts).

**Experimental Protocol**

The protocol was initiated 30 minutes after surgical preparation was completed by applying a 100 μA continuous anodal direct current to the stimulation electrode in the LCCA. Current was delivered from a 9-V nickel-cadmium battery connected in series by a 250,000-Ω potentiometer to the intraluminal coronary artery electrode. The electrical circuit was completed by placing the cathode in a subcutaneous site. If a complete occlusive thrombus was not established within 3 hours, the animal was excluded from the study. If occlusive coronary artery thrombosis occurred, as detected by 0 ml/min CBF and ST segment elevation on limb lead II of the electrocardiogram, then electrical stimulation was discontinued after 30 minutes and thrombolytic treatment was initiated.

Intravenous rt-PA was administered as a 1-mg/kg bolus followed by the remainder of a total dose of 25 mg as a continuous infusion over the subsequent hour. If thrombolytic therapy was successful in reestablishing CBF, then within 5 minutes, a bolus injection of 0.8 mg/kg 7E3 F(ab')2, or the saline diluent was administered in a randomized and blinded fashion. Spontaneous oscillations in CBF often occurred preceding rethrombosis, and the number of these oscillations was noted. Each experiment was considered complete 3 hours after initial thrombolysis. When obvious lysis did not occur during the subsequent 3 hours, the animals were not randomized to receive either 7E3 F(ab')2, or the saline diluent.

At the conclusion of the experimental procedure, 10 ml Super Impeze Blue dye was injected through the left atrial cannula while occluding the LCCA just proximal to the area where the stimulating electrode was inserted. In this manner, the area perfused by the LCCA, based on the fact that no dye can enter this distribution, was determined as the in vivo area at risk (AR). The heart was fibrillated electrically 10 seconds later and removed quickly. The LCCA was dissected free as far as possible distal to the site of thrombosis and opened longitudinally; the intracoronary position of the implanted stimulation electrode was verified, and the thrombus was removed and weighed. The heart was cut from apex to base in 1.0-cm-thick sections, which were incubated in triphenyltetrazolium chloride (TPT) for 5 minutes at 37°C. The transverse sections were weighed and traced onto clear plastic; the area stained by Super Impeze Blue dye was the nonrisk region, and the red stained tissue was considered viable AR, whereas tissue that remained pallid was considered infarcted (INF). Quantifying infarct size with TPT separates viable from nonviable myocardial tissue as detected by histochemical reactions between TPT and dehydrogenase enzymes. The various demarcated areas were quantified with an Apple IIE microcomputer and the data were expressed as a percentage of the AR and of the total left ventricular (LV) weight.

**Platelet Studies**

Blood (20 ml) was withdrawn from the internal jugular cannula into a plastic syringe containing 3.2% sodium citrate as the anticoagulant (1:10 citrate/blood [vol/vol]) at baseline, immediately on thrombolysis, and 1 and 3 hours after treatment with either 7E3 F(ab')2, or saline diluent. The platelet count was determined with a Haema Count MK-4/HC system (J.T. Baker, Allentown, Pennsylvania). Platelet rich plasma (PRP), the supernate present after centrifuging anticoagulated whole blood at 1,000 rpm for 5 minutes (140g), was diluted with platelet poor plasma (PPP) to achieve a platelet count of 200,000/mm³. PPP was prepared after the PRP was removed by centrifuging the remaining blood at 12,000g for 10 minutes and discarding the bottom cellular layer. Ex vivo platelet aggregation was measured by established spectrophotometric methods with a four-channel aggregometer (BioData-PAP-4, BioData Corporation, Hatboro, Pennsylvania) by recording the increase in light transmission through a stirred suspension of PRP maintained at 37°C. Aggregation was induced with ADP (5 μM) and arachidonic acid (AA) (0.65 mM). Epinephrine (550 nM) was used to prime the platelets before AA stimulation. Values were expressed as percentage of aggregation, which represented the percentage of light transmission standardized to PRP and PPP samples yielding 0% and 100% light transmission, respectively.

**Coagulation Studies**

Citrate-anticoagulated plasma was used to study the prothrombin time (PT), activated partial thromboplastin time (PTT), and the fibrinogen concentration at baseline, immediately on thrombolysis, and 1 and 3 hours after treatment with either 7E3 F(ab')2, or saline diluent. To assess single or combined deficiencies of clotting factors of the extrinsic coagulation pathway (factors VII, X, and II [prothrombin]), as well as V and I [fibrinogen], the PT was determined with thromboplastin C (dried rabbit...
brain thromboplastin with calcium). To evaluate abnormalities in the enzymes and cofactors of the intrinsic coagulation pathway necessary for prothrombin activation, the PTT was determined using calcium chloride (0.02 M) and activated cephaloplastin reagent (liquid rabbit brain cephalin with plasma activator).  

The PTT is especially sensitive to functional deficiencies of factors VIII, IX, XI, XII, prekallikrein (Fletcher factor), and high molecular weight kininogen (Fitzgerald factor), and will also detect severe functional deficiencies in factors II, V, X, or I. Fibrinogen concentrations were determined with thrombin reagent (lyophilized bovine factor), and Owren’s veronal buffer (0.0284 M sodium barbital in 0.125 M NaCl, pH 7.35). The clotting time obtained was compared with that of a standardized fibrinogen preparation. Control plasma for these assays was a lyophilized preparation of human plasma (Ci-Trol Coagulation Control, Level 1). Fibrin and fibrinogen degradation products (FDP), caused by digestion of fibrinogen or fibrin by plasmin or other proteolytic enzymes, were detected with immunological methods observing the clumping of Latex Anti-Fibrinogen (0.6% suspension of polystyrene latex particles coated with rabbit antihuman fibrinogen in buffer) in Sorensen’s glycine buffer (0.1 M glycine and 0.1 M saline, pH 8.2).  

Sample tubes for FDP assays contained thrombin (20 NIH units) and soybean trypsin inhibitor (approximately 3,600 NF units).

Statistical Analysis

The data are expressed as the mean±SEM. The groups were compared using one-way analysis of variance (ANOVA). If a significant $F$ resulted, the Newman-Keuls multiple range test was applied to determine where differences were located among the groups or subpopulation means. When indicated, parameters were analyzed by two-factor repeated measures ANOVA. If a significant interaction was present, a Bonferroni multiple comparison method was used to determine the time period(s) at which the treatment means differed significantly. If the time factor main effect was significant, a Newman-Keuls multiple comparison test was used to determine which means were significantly different. Stepwise multiple linear regression analyses were performed to determine if 1) any baseline coagulation study or platelet count could predict time to thrombosis, 2) any baseline or time of lysis coagulation study or platelet count could predict time to thrombolysis, and 3) any baseline, time of lysis, 1-hour or 3-hour coagulation study, or platelet count could predict rethrombosis. A $p$ value less than 0.05 was considered significant.

Results

Group Characteristics

Thirty-three dogs were entered into this experimental protocol. Eight dogs were excluded before the thrombolytic agent was given; four dogs failed to develop thrombotic occlusion in less than 3 hours, one developed spontaneous occlusion before application of the anodal current was initiated, and three dogs fibrillated at the time of thrombotic occlusion (before randomization). The remaining 25 dogs developed thrombotic occlusion of the LCCA in a predictable time frame. In six dogs, however, the administered intravenous dose (25 mg) of single-chain rt-PA was not an effective thrombolytic agent, and detectable CBF was not reestablished. Data regarding these animals will be presented separately. Nineteen experiments were completed successfully. The animals were assigned randomly in a blinded fashion to one of two treatment groups, group 1, control (saline diluent, $n=10$) or group 2, murine monoclonal antiplatelet antibody 7E3 [7E3 F(ab’)$_2$, $n=9$]. There was no difference between the two groups in the weight of the dogs (group 1, 16.1±0.7 kg; group 2, 16.5±0.6 kg), the weight of the hearts postmortem (group 1, 128±8 g; group 2, 129±5 g), or percentage of total left ventricle perfused by the LCCA (group 1, 35±3%; group 2, 34±4%). The two groups did not differ with respect to hemodynamic variables recorded at the beginning of the experimental protocol (i.e., heart rate, blood pressure, and coronary blood flow).

Thrombosis Data

The time required for occlusive coronary artery thrombosis to occur was similar to both groups (Figure 1). The time required for reperfusion to occur, that is, for the thrombolytic treatment to be effective, was also similar. The 7E3 F(ab’)$_2$ was given after the rt-PA, at the time of lysis, so it would not have any effect on the initial efficacy of the thrombolytic agent. The mean time to rethrombosis, when it occurred, was similar in both groups. All the control animals ($n=10$) in group 1 experienced rethrombosis, whereas only two of the nine 7E3 F(ab’)$_2$-treated animals developed rethrombosis ($p<0.05$). The thrombus weight determined 3 hours after thrombolysis was larger in group 1 than group 2 (7.0±2.3 vs 1.5±0.7 mg, respectively, $p<0.05$). After

![Figure 1. Bar graph of overview of coronary artery thrombosis, thrombolysis, and rethrombosis. There were no significant differences between first two groups with respect to time required for occlusive intracoronary thrombus to form, rt-PA to induce thrombolysis with restoration of coronary blood flow, or time required for rethrombosis to occur. Incidence of rethrombosis, however, was higher in control group (10/10) than the 7E3 F(ab’)$_2$-treated group (2/9, $p<0.05$). Data are presented as mean±SEM.](image-url)
were found in the two arteries that rethrombosed (1.2 mg and 2.5 mg) as determined by the absence of CBF recorded with an electromagnetic flow probe placed on the LCCA.

**Left Circumflex Coronary Artery Blood Flow**

At the initiation of each experiment, CBF was similar (Figure 2). When thrombolysis occurred, the initial reperfusion blood flow was similar in all the animals before being assigned randomly to one of the two treatment groups. During the 3 hours after thrombolysis, CBF decreased in both groups. The 7E3 F(ab')2-treated animals with patent vessels had a final CBF of 13.4±1.6 ml/min (n=7), which was higher than the value in the control group, where it decreased progressively in each animal to a negligible level as occlusive intravascular thrombus reformed. When CBF diminished in the control group, there were spontaneous hyperemias (oscillations) during which blood flow decreased to near zero and then abruptly and spontaneously resumed at a level higher than the preceding flow (Figure 3). These oscillations only occasionally occurred after 7E3 F(ab')2 treat-

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**FIGURE 2.** Graphic plot of left circumflex coronary artery blood flow (LCCA). Baseline and reperfusion (noted 5 minutes after thrombolysis) LCCA blood flows were similar in both groups. Rethrombosis occurred in all control animals (n=10) and two of animals given 7E3 F(ab')2 (n=9). There was significant decline in LCCA blood flow over time in both groups (p<0.05), which was most apparent at final determination (3 hours after thrombolysis). At that time, LCCA blood flow was lower in control animals than 7E3 F(ab')2-treated animals (*p<0.05). Data are presented as mean±SEM.

**FIGURE 3.** Representative tracings during protocol in control animal. From top to bottom, four channels display 1) limb lead II electrocardiogram (ECG), 2) systemic blood pressure, 3) mean left circumflex coronary artery (LCCA) blood flow, 4) phasic LCCA blood flow. From left to right, four times recorded are baseline (A), thrombosis (B), thrombolysis (C), and rethrombosis (D) with oscillations. At the time of thrombosis, there is ECG evidence of transmural ischemia with ST segment elevation (8mV) and modest decrease in systemic blood pressure. LCCA blood flow is 0 ml/min. During early thrombolysis, ST segment elevation has been replaced by inverted T waves without Q wave evidence of infarction, and blood pressure has returned to baseline level. Reperfusion LCCA blood flow is higher than baseline. When LCCA blood flow is decreasing and rethrombosis is impending (D), there are numerous ectopic beats (downward deflections on ECG). When rethrombosis occurs, these ectopic beats tend to disappear and LCCA blood flow is negligible. Oscillations are characterized by gradual decrease in blood flow to near zero followed by abrupt and spontaneous restoration in blood flow. During this oscillating blood flow pattern, ectopic beats are frequent and accompany reperfusion.
Table 1. Coagulation and fibrinolytic profiles

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<th>Baseline</th>
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<td>7E3 F(ab')$_{2}$</td>
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<td>7E3 F(ab')$_{2}$</td>
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<td>1,134±568</td>
<td>1,192±634</td>
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<td>Platelet counts (platelets/mm$^3$×10$^9$)</td>
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Changes over time were significant (*p<0.01, †p<0.05).
Difference between groups at one time significant (†p<0.02, §p<0.01, ||p<0.05, ¶p<0.001).

ment (group 1, 5.2±0.9 oscillations; group 2, 0.7±0.4 oscillations; p<0.05).

Hemodynamic Responses

There were no differences at any time between the two groups with respect to heart rate or mean arterial blood pressure (MAP). In both groups, the heart rate was unchanged over the course of the protocol. The MAP tended to decrease at the time of LCCA thrombosis (group 1, 108±8 to 99±3 mm Hg; group 2, 92±4 to 86±3 mm Hg). In group 1, MAP on reperfusion was 89±5 mm Hg, which decreased to 80±7 mm Hg after 3 hours because all animals developed coronary artery rethrombosis. In group 2, MAP on reperfusion was 86±6 mm Hg, and when the arteries remained patent during the subsequent 3 hours, MAP was 83±6 mm Hg (n=7). The two animals in group 2 that developed coronary artery rethrombosis experienced a decrease in MAP (56±7 mm Hg). In these two cases, CBF and MAP were declining slowly at the same rate, and it was unclear whether the hypotension and decreased coronary perfusion precipitated rethrombosis.

Platelet Studies

The platelet counts were not different between the two groups at any time; however, they tended to decrease during each experiment (Table 1). The platelet aggregation studies were similar at baseline for all animals, and the AA-induced aggregation in the control group was unchanged over the course of the protocol. The ADP-induced aggregation in both groups diminished by 50% after rt-PA (p<0.05) (Table 1). There was a direct correlation detected between the fibrinogen concentration and the ADP-induced platelet aggregation at the time of thrombolysis (p<0.05), which persisted in the control group 3 hours after the rt-PA infusion. One hour after the 7E3 F(ab')$_{2}$ injection, there was a further significant diminution in aggregation with both agonists, AA and ADP, as compared with aggregation at baseline and at the time of thrombolysis. This inhibition of aggregation by 7E3 F(ab')$_{2}$ persisted throughout the course of the experiment.

Coagulation Profiles

At the beginning of each experiment, coagulation studies including PT, PTT, FDP, and fibrinogen levels were similar in all the animals (Table 1). There was a prolongation in both the PT and PTT after the rt-PA. The prolongation of the PT normalized more slowly in group 2 than group 1, and the difference reached significance 3 hours after clot lysis (p<0.02). The PTT was prolonged to a greater extent in group 2 than group 1 at the time of lysis, but by 1 hour after lysis, the values in the two groups tended to normalize. The fibrinogen level decreased after the rt-PA in both groups (p<0.01). There was a tendency for the concentration to increase over time. In the animals given 7E3 F(ab')$_{2}$, however, this occurred more slowly than in the control group, so that the plasma fibrinogen concentration 3 hours after lysis was lower
Myocardial Infarct Size

The AR was determined in vivo and was expressed as a percentage of the total LV perfused by the LCCA (AR/LV), which was similar in both groups (Figure 4). The infarct size expressed as a percentage of the area at risk (INF/AR) or as a percentage of the total left ventricle (INF/LV) was smaller in the 7E3 F(ab')2-treated group than the control group ($p<0.05$). The duration of ischemia, because of failure to maintain demonstrable reperfusion, was longer in the control group than the 7E3 F(ab')2-treated group (group 1, 130±9 min; group 2, 47±15 min; $p<0.05$). The prolonged ischemia that occurred because of failure to sustain reperfusion in group 1 might have contributed to the extension of myocardial injury observed in this group.

Discussion

During the initial stages of myocardial infarction, effective thrombolytic therapy has been shown to improve myocardial function and survival. There have been difficulties, however, in finding an ideal thrombolytic regimen that is not compromised by either hemorrhagic complications or early acute rethrombosis. Because physiologic hemostasis must be maintained, there is a limit to the amount of thrombolytic agent that can be used to achieve initial arterial patency and prevent acute reocclusion. To overcome this problem, several adjunctive therapies have been used with thrombolytic agents. Clinically, the use of aspirin, dipyridamole, calcium channel blockers, heparin, or nitrates have had little impact on the 20–30% acute rethrombosis rate with either streptokinase or rt-PA. The model described in this study uses electrical induction of vessel wall injury as a means of promoting intracoronary artery thrombosis in the presence of a stenosis. The experimental models appears to mimic the clinical situation with respect to a spontaneously developing thrombus in response to endothelial injury. The stenosis in this experimental model is a modest coronary artery lesion, not a critical lesion. The cross-sectional area of the induced stenosis is greater than the 0.4-mm² cross-sectional area thought by Harrison et al. to be, itself, a risk for rethrombosis because of local flow turbulence. The results of several clinical trials, including the TIMI-II-B trial, would suggest that coronary artery stenoses might not be critical after thrombolysis and do not require angioplasty because remodeling occurs, leaving a residual luminal area that does not result in functionally significant ischemia. The experimentally induced thrombus is platelet rich and occurs after intimal injury that has stimulated endogenous platelets, leukocytes, and coagulation factors. Similarly, acute catheterization studies in patients with unstable angina or myocardial infarction have shown intracoronary thrombus in most cases. The clinically observed thrombi contain numerous platelets, and there is evidence that circulating platelets can be

![Figure 4. Bar graph showing myocardial infarct size. Area of left ventricle (LV) perfused by left circumflex coronary artery (LCCA) comprised in vivo area at risk (AR) for infarction (INF). In both groups, AR/LV was similar. In those animals that received 7E3 F(ab')2, LCCA did not rethrombose as often as it did in control group, and consequently, infarct size (INF/AR and INF/LV) was smaller (*p<0.05). Data are presented as mean±SEM.](http://circ.ahajournals.org/content/circulation/97/18/2421/F4.large.jpg)
activated in the diseased arterial segment, causing increases in transmyocardial thromboxane concentrations.33–35 When the circulating platelets are activated but not enmeshed in the thrombus, they can form small intramyocardial aggregates, which Davies et al36 have described in patients with unstable angina who experienced sudden ischemic cardiac death. Additionally, there have been recent reports suggesting even greater platelet activation during the infusion of thrombolytic agents, that is, streptokinase and rt-PA.37,38

Platelet aggregates interacting with a damaged endothelial surface or exposed subendothelial component are a major factor early in coronary artery thrombosis and subsequent rethrombosis.39 The release of thromboxane and serotonin from the activated platelets is one mechanism proposed as a cause of reocclusion.7 In vivo, platelet aggregation depends on binding of fibrinogen to the GPIIb/IIIa receptor.40 Blocking GPIIb/IIIa with the murine monoclonal antiplatelet antibody 7E3 [7E3 F(ab')2] prevents platelet aggregation and, therefore, acute rethrombosis after lytic therapy can be modified. There are several previously published reports on 7E3 F(ab')2 as an adjunct preceding the rt-PA infusion that induced coronary artery thrombolysis. Studies in a canine preparation with localized thrombin-induced clot in the externally traumatized left anterior descending coronary artery with a critical stenosis showed that 7E3 F(ab')2 facilitated initial thrombolysis and prevented rethrombosis.11,12 In these studies, the initial thrombin-induced clot is not platelet rich. The total dose of rt-PA given to each animal varied (0.225 or 0.45 mg/kg bolus every 15 minutes up to four doses11 or 15–30 μg/kg/minute for 30–60 minutes12), and other agents (e.g., intravenous heparin, lidocaine, and procainamide) were administered. Fitzgerald et al41 recently reported that 7E3 F(ab')2 shortened the time to reperfusion and prevented reocclusion in a long-term sedated canine model of LCCA occlusion induced by electrical injury (200 μA) similar to the procedure used in the present study. The 7E3 F(ab')2 inhibited platelet aggregation but did not prevent platelet activation as detected by urinary excretion of 2,3-dinor-thromboxane B2. Again, the total dose of rt-PA given to each animal varies (10 μg/kg/min until 10 minutes after lysis) and rt-PA was not administered until 2 hours after thrombosis. The persistent severe stenosis (80–90%) described represents thrombus and eschars from the electrical injury. The major differences in these studies as compared with our study were that we 1) administered the 7E3 F(ab')2 after thrombolysis, 2) infused the same total dose of rt-PA in each animal, 3) used a model with minimal endothelial electrical injury, 4) did not have a critical stenosis initially nor did one persist after lysis, and 5) did not administer other pharmacologic agents so that infarct size could be determined at the conclusion of the study.

In this study, two dogs in the 7E3 F(ab')2 treatment group developed nonocclusive thrombi, which did not diminish CBF significantly below that determined in arteries without detectable thrombi. By preventing or minimizing aggregation and limiting the local concentration of platelet-derived vasoconstrictive substances, the 7E3 F(ab')2 stabilized reperfusion coronary blood flow even when a small thrombus was present in the coronary artery. Recent studies have implicated intracoronary platelet deposition as a cause of ultraspinically measurable, intense constriction of epicardial coronary arteries in vivo. Platelet-derived mediators, serotonin and thromboxane, can trigger this event because specific receptor antagonists modify the vasoconstriction and the ensuing cyclic flow variations in canine coronary arteries.42 Of interest, the thromboxane receptor antagonist promoted coronary vasodilatation in the manipulated coronary arterial segment, whereas the serotonin receptor antagonist did not. Similarly, after rt-PA induced thrombolysis, platelet activation and thromboxane are important mediators of vasomotor instability and reocclusion.7,41 Although neither heparin7,12 nor aspirin12,41 completely prevented reocclusion, intravenous aspirin did appear to facilitate reperfusion.

There were two animals in the 7E3 F(ab')2 treatment group that developed r ethrombosis in the presence of very small intracoronary thrombi. This suggests that either the entire thrombus was not retrieved or that the size of the thrombus did not always correlate with reocclusion. It is unlikely that part of the thrombus was lost distal to the endothelial lesion because parts of the thrombi were firmly adherent to the vessel wall, and in the dissection, a long segment of the artery was removed intact before any attempt was made to localize and isolate the thrombus. Thus, in some cases, factors other than the presence of a small thrombus can determine coronary artery blood flow patterns. Marcus et al43 have demonstrated in vitro that aspirin-treated platelets can release AA, which serves as a source for neutrophil leukotriene production that, in turn, can alter coronary vasomotor tone. It is uncertain whether the local decrease in platelet accumulation, or the inhibition of aggregation by 7E3 F(ab')2, or both prevent all mediator release and biochemical cell-cell interaction through shared precursors of the eicosanoid pathway. It might be that neutrophils and endothelial cells interact through platelet-derived AA, and this explains the observed cessation of coronary blood flow in the presence of small thrombi.

Administration of the 7E3 F(ab')2 antibody was not associated with significant changes in the circulating platelet count or measured hemodynamic variables. There was a decrease in the platelet count in the control group, however, perhaps because platelets were being consumed in the site of the thoracotomy incision, the intracoronary thrombus, or the infarcting myocardium. Whenever rethrombosis occurred in the control group, it was accompanied by a modest decrease in MAP. The two 7E3 F(ab')2-treated animals that developed r ethrombosis also experienced hypotension, but this occurred at a point
in time considerably removed from the antibody administration. A similarly benign response to the F(ab')2 fragments has been confirmed in other models using various animal species.11,41,44,45

Coincident with the presence of the 7E3 F(ab')2 on the GPIIb/IIIa receptor is the observation that ex vivo inhibition of platelet aggregation induced with either ADP or AA persisted for 3 hours after the antibody had been infused. During this time, there was no obvious increased bleeding or changes in hemodynamic variables to suggest increased internal blood loss from the animals in group 2 as compared with the control group. Other investigators using the same 7E3 platelet antibody have observed an even longer duration (up to 48 hours) of inhibition of platelet aggregation with minimal bleeding complications.45

Initial thrombosis occurred at 34±5 minutes in group 1 and 25±5 minutes in group 2, after the intravenous bolus injection of rt-PA while it was still being infused (1 mg/kg bolus followed by the remainder of a total dose of 25 mg as a continuous infusion over the subsequent hour), but before the 7E3 F(ab')2 antibody was administered. At this time, there was a decrease in platelet aggregation in response to ADP. Coincidently, the fibrinogen concentrations for each group had reached their nadir (group 1, 36% of baseline fibrinogen; group 2, 13% of baseline fibrinogen). During the subsequent 3 hours, ADP-induced platelet aggregation remained abnormal in the control animals (group 1) although fibrinogen concentrations rebounded to 58% of the baseline value. The fibrin and FDP remained increased to approximately the same extent throughout this time. In vitro, rt-PA has been shown to promote platelet disaggregation after ADP-induced aggregation. It is presumed that the disaggregation occurs because of proteolysis of cohesive fibrinogen.46 The rt-PA was reported not to alter platelet surface glycoproteins IIb/IIIa or glycoprotein Ib, nor did FDP affect platelet aggregates. Unfortunately, in the study reported herein, even when rt-PA induced lysis in vivo and partially inhibited ex vivo platelet aggregation in response to ADP, rethrombosis of the coronary artery occurred in all animals in the control group within an hour (50±9 minutes). At this time, the rt-PA infusion had been stopped for approximately 30 minutes.

There is an overall suggestion that the animals randomized to 7E3 F(ab')2 had a greater systemic lytic effect from the rt-PA infusion at the time of lysis, that is, lower fibrinogen levels and higher FDP. The results of the coagulation studies were unknown at the time of randomization, and were not clinically detectable as increased spontaneous blood loss or oozing. Interestingly, the group of dogs that never developed reperfusion during the rt-PA infusion had 1) the highest nadir in fibrinogen (144±85 mg/dl [39% of baseline]), 2) FDP level intermediate between the other two groups (624±476 µg/ml), 3) inhibition of ADP-induced platelet aggregation (45±10% to 16±11%, p<0.05). It seems that a significant and prolonged lowering in plasma fibrinogen concentration is important for coronary artery thrombolysis and prevention of reocclusion.

It might be premature to suggest that infarct size is limited in the 7E3 F(ab')2-treated animals because infarct size determined less than 24 hours after the onset of ischemia might not detect all the nonviable myocardium. Speculation exists that postmortem comparison of infarct size between the two groups subjected to a similar experimental protocol provides a meaningful assessment of myocardial injury although it might not reflect the full extent of ischemic damage.14–16 In this study, the myocardium at risk, that area perfused by the proximal LCCA in vivo, was similar in both groups (~35±4%). Because the 7E3 F(ab')2 was effective in preventing rethrombosis, the observed duration of ischemia was short, and the resulting infarct size was small. If thrombolytic efficacy is improved and rethrombosis is prevented so that the duration of ischemia is minimal, then 7E3 F(ab')2—treatment has the potential for serving as an adjunct to the clinical application of thrombolytic therapy.

The intravenous administration of the murine monoclonal 7E3 F(ab')2 platelet antibody did not produce any untoward effects on the recorded cardiovascular variables or the circulating platelet count. Neither bleeding, excessive blood loss, nor hemodynamic instability was a problem during this series of experiments. Although the antibody did not completely prevent rethrombosis, it did result in a significant decrease in the intracoronary thrombus mass. Whether or not the immunogenicity of murine monoclonal antibody will preclude its clinical application was not assessed in this study. Based on these experimental observations, it might be suggested that the 7E3 F(ab')2 platelet antibody will serve as an adjunct to measures directed against intravascular thrombus formation, particularly those associated with acute coronary artery thrombosis after percutaneous transluminal coronary angioplasty or rethrombosis after successful thrombolytic therapy.

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