Published by Elsevier Inc.
ISSN 0735-1097/04/$30.00
doi:10.1016/j.jacc.2003.08.039

Short-Term Intravenous Eptifibatide Infusion Combined With Reduced Dose Recombinant Tissue Plasminogen Activator Inhibits Platelet Recruitment at Sites of Coronary Artery Injury

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OBJECTIVES This study was designed to determine in a dog model of coronary thrombosis whether short-term eptifibatide (Ep) combined with low-dose plasminogen activator (rt-PA) inhibits platelet recruitment at sites of endothelial damage after normalization of platelet function.

BACKGROUND Ep plus reduced-dose rt-PA has not previously been shown to render a recanalized coronary artery resistant to platelet recruitment after normalization of platelet function.

METHODS Inhibition of platelet recruitment was studied by scanning electron microscopy (SEM) in a canine model of left anterior descending (LAD) thrombosis. In phase I treatment groups were: 1) Ep (n = 6); 2) Ep + rt-PA (n = 6); 3) rt-PA (n = 6); and 4) placebo (n = 4). Coronary blood flow was monitored and LAD segments excised for SEM after 90-min infusion of study drug. In phase II, dogs were randomized to Ep alone (n = 5) or to Ep + rt-PA (n = 5). Coronary blood flow was monitored during and 120 min after cessation of drug when platelet function had returned to normal and LAD segments were excised.

RESULTS All animals except placebo showed reflow. In phase I, SEM showed an absence of platelet aggregates with Ep alone and with Ep + rt-PA, but not with rt-PA alone. In phase II, SEM showed an intimal surface devoid of mural thrombus and platelet aggregates only in Ep + rt-PA treated arteries. Ep-alone treated arteries showed new platelet aggregates at sites of residual mural thrombus.

CONCLUSIONS Short-term infusion Ep plus low-dose rt-PA acutely neutralizes the ability of damaged endothelial surfaces to recruit new platelets by inhibiting platelet aggregation and eliminating residual mural thrombus. (J Am Coll Cardiol 2004;43:287–94) © 2004 by the American College of Cardiology Foundation

The pathophysiologic event initiating acute myocardial infarction (MI) is most often rupture of a vulnerable atherosclerotic plaque, leading to formation of an occlusive intraluminal thrombus (1–3). At the site of endothelial disruption, there is immediate adherence and deposition of activated platelets on the injured arterial surface (4). The progression of mural to occlusive thrombus depends upon the thrombogenicity of the damaged surface, the capacity of the endogenous fibrinolytic system to limit thrombus formation, and the severity of the stenosis (5,6). Although exogenous plasminogen activators, in conjunction with aspirin and heparin, are widely used to restore flow, they do not fully address the thrombogenicity of the damaged arterial surface. The exposed subendothelial structures and the reservoir of thrombin within the residual mural thrombus provide the substrate for recurrent thrombus formation at the site of arterial damage (7–9). The reaccumulation of platelet-rich thrombus on this substrate predisposes to reocclusion and recurrent ischemic events (10–12). The neutralization of these residual thrombogenic stimuli is likely required to maintain coronary patency following successful thrombolysis for acute MI.

Our previous studies in a canine model of coronary thrombosis have demonstrated that platelet glycoprotein (GP) IIb/IIIa receptor blockade using the 7E3 antibody in combination with low-dose plasminogen activator accelerates reflow and prevents coronary reocclusion (10,13–15). The prevention of reocclusion in this model required platelet inhibition with >80% GP IIb/IIIa receptor blockade. However, because of the prolonged blockade of the GP IIb/IIIa receptor by the 7E3 antibody, we could not determine whether failure of the damaged arterial endothelium to recruit new platelets was a consequence of acute neutralization of the thrombogenic surface or simply a result of persistent receptor blockade.

Eptifibatide (Ep) is a reversible platelet GP IIb/IIIa receptor antagonist with a short half-life. In the first phase of the present study, we investigated the efficacy of a 90-min continuous intravenous infusion of Ep alone and in combination with reduced-dose recombinant tissue plasminogen

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Manuscript received June 19, 2002; revised manuscript received July 10, 2003, accepted August 26, 2003.

Abbreviations and Acronyms

ACT = activated clotting time
Ep = eptifibatide
GP = glycoprotein
IV = intravenous
LAD = left anterior descending
MI = myocardial infarction
rt-PA = tissue plasminogen activator
SEM = scanning electron microscopy

activator (rt-PA) to: 1) induce reflow with complete lysis of intraluminal and mural thrombus, 2) inhibit the recruitment of platelets onto the damaged endothelial surface, and 3) prevent coronary reocclusion in the presence of high-grade coronary stenosis. In the second phase, we determined: 1) whether a short-term infusion of Ep alone would acutely neutralize vessel wall thrombogenicity (in the presence of residual mural thrombus), or 2) whether the addition of low-dose exogenous plasminogen activator to Ep would be required to eliminate residual mural thrombus in order to completely neutralize vessel wall thrombogenicity. The purpose of this study was to determine whether short-term Ep in concert with reduced-dose plasminogen activator could acutely prevent platelet recruitment, new thrombus formation, and reocclusion at sites of endothelial damage despite the return of platelet function to normal.

METHODS

Phase I

Observations during Ep infusion. Animals used in this study were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, revised 1996). A total of 22 adult mongrel dogs (20 to 25 kg) were anesthetized with intravenous sodium pentobarbital (35 mg/kg), intubated, and artificially ventilated. A left thoracotomy was performed, and the internal mammary artery was cannulated for continuous blood pressure monitoring. The experimental preparation, consisting of occlusive left anterior descending (LAD) coronary artery thrombosis with endothelial damage and superimposed distal stenosis, was prepared as previously described (10). Briefly, the isolated LAD segment was traumatized by four consecutive external compressions with blunt forceps during 3 to 5 s. Snare occlusions were made distal to the flow probe and proximal to the constriction site. Thrombin, 0.1 ml of 100 U/ml (Thrombinar, Armour Pharmaceutical, Kankakee, Illinois), mixed with 0.3 ml blood, was injected through the side branch catheter into the emptied coronary artery segment. After 5 min the proximal snare was released; 2 min later, the distal tourniquet was released. Total occlusion of the artery was demonstrated by ultrasonic flow probe. Twenty minutes after formation of a stable, occlusive erythrocyte-rich coronary artery thrombus, heparin (3,000 U) and aspirin (Aspegic, 10 mg/kg) were administered by intravenous (IV) bolus injection. Upon persistence of occlusion, experimental treatment was initiated 10 min later. The initial coronary occlusion and subsequent reflow in the artery were documented and monitored continuously by an electromagnetic flow probe during the infusion of the study drug.

Intravenous infusions were performed with a constant rate infusion pump. Additional 1,000 U bolus injections of heparin were administered as needed to maintain the activated clotting time (ACT) >250 s. Reflow time was defined prospectively as the interval between the initiation of eptifibatide (Group I), rt-PA (Groups II and III), or placebo (Group IV) therapy and the recurrence of blood flow. Baseline flow was measured after placement of the external constrictor and before total occlusion of the artery. Reflow was defined as the return of >25% of baseline blood flow. Reocclusion was defined as the reduction of coronary blood flow to <25% of baseline. Coronary artery patency status was categorized as follows: 1) persistent patency: persistent flow without reocclusion after initial reflow; 2) cyclic reflow and reocclusion: alternating cycles of reocclusion after initial reflow; 3) persistent occlusion: no recurrence of blood flow.

Treatment. Initial dose-finding studies determined that IV Ep administration of 0.5 mg/kg of body weight followed by a continuous infusion of 6 μg/kg/min for 90 min was the minimally effective dose to inhibit completely adenosine diphosphate-induced platelet aggregation ex vivo when the sample was collected in heparin. rt-PA was administered as a 0.45 mg/kg of body weight IV bolus (1/4 of the full canine thrombolytic dose) every 15 min until reflow, not exceeding a maximum of four injections. A comparative study of the following treatments for coronary thrombolysis was then performed: Group I) eptifibatide alone, n = 6; Group II) eptifibatide + rt-PA, n = 6; Group III) rt-PA alone, n = 6; Group IV) placebo (IV saline bolus plus continuous infusion), n = 4.

Hemostasis analysis. Platelet counts and hematocrit measurements were determined on blood samples collected into 0.05 ml (15%) liquid ethylenediaminetetraacetate (K3 EDTA) at baseline and immediately before cessation of therapy at 90 min. Activated clotting time values were determined on 1 ml whole blood samples in a Hemochron analyzer. Blood samples (10 ml) for platelet aggregation were collected into 4 U/ml heparin containing 150 KIU/ml aprotinin at pretreatment, 10, 30, and 60 min after start of treatment, and immediately before cessation of infusion at 90 min. Platelet-rich plasma was prepared and tested for platelet aggregation induced by a threshold concentration of ADP (Sigma Diagnostics, St. Louis, Missouri) in a dual-channel aggregometer (Chrono-log Corp., Havertown, Pennsylvania) at 37°C with constant stirring, as previously described (16). Quantitative analysis of aggregation was determined as the maximal percent change in transmittance

Table 1. Reflow and Patency Status

Group	Agent	Reflow		Patency Status†			Pathology
		N/total N	Time (min)	PO	CR	PP	
I	Ep	6/6	42 ± 28*	—	—	6	MT,ER
II	Ep + rt-PA	6/6	5 ± 3*	—	—	6	Clean
III	rt-PA	6/6	20 ± 17	—	4	2	MT,PR
IV	Saline	0/4	—	4	—	—	OT

*p = 0.002; †p = 0.001. Values are mean ± SD.
CR = cyclic reflow; Ep = eptifibatide; ER = erythrocyte-rich; MT = mural thrombus; OT = occlusive thrombus; PO = persistent occlusion; PP = persistent patency; PR = platelet-rich; rt-PA = recombinant tissue plasminogen activator.

(% delta T), measured from baseline to the lowest portion of the curve, after addition of agonist. Template bleeding times were measured at baseline, 30, and 60 min after the start of therapy, and immediately before cessation of infusion at 90 min, as previously described (15).

Pathologic examination. At the end of the experiment, dogs were euthanized with an overdose (100 mg/kg IV) of pentobarbital. The LAD coronary artery was then perfusion fixed in situ with 5% buffered formaldehyde. The thrombosed, stenotic, and poststenotic segments, as well as the entire distal segment of the LAD, were then removed intact and preserved. LAD segments were post-fixed in 4% glutaraldehyde overnight. Specimens were then dehydrated, critical point dried, mounted individually, and examined by scanning electron microscopy (SEM). Micrographs were interpreted by a cardiac pathologist, blind to the treatment protocols.

Statistical analysis. Results are expressed as mean values ± standard deviation (SD). The significance of differences between groups was determined by Student *t* test for unpaired values. A paired *t* test was used for determination of intragroup differences. Fisher exact test was used to compare the significance of differences in the frequencies of reflow and reocclusion in the four treatment groups.

Phase II

Observations after cessation of Ep infusion. After the initial 22 experiments, 10 additional dogs were assigned to receive either Ep + rt-PA (n = 5) or Ep alone (n = 5). Experimental procedures were performed as described earlier, but coronary blood flow was monitored continuously for an additional 120 min after cessation of the Ep infusion. Heparin continued to be administered as needed to maintain an ACT >250 s. ADP-induced platelet aggregation was measured at baseline, 10 min after initiation of eptifibatide therapy, immediately before cessation of infusion at 90 min, and then at 30, 60, 90, and 120 min after discontinuation of Ep infusion.

Venous blood samples (3 ml) for measurement of plasma Ep levels were collected into 0.05 ml (15%) liquid EDTA at baseline, 30, 45, 60, and 90 min after initiation of Ep and then at 30, 60, 90, and 120 min after cessation of infusion.

The thrombosed, stenotic, and poststenotic segments, as well as the entire distal segment of the LAD from all 10 animals, were removed intact and preserved. The LAD segments were post-fixed in 4% glutaraldehyde overnight. Specimens were then dehydrated, critical point dried, mounted individually, and examined by SEM. Micrographs were interpreted by a cardiac pathologist blinded to the treatment protocols.

RESULTS

Phase I

Observations during Ep infusion. Experiments were first performed to determine whether Ep enhanced the ability of rt-PA to lyse thrombi. The results for coronary artery blood flow and patency status in the initial 22 dogs are presented in Table 1. All animals, except those given placebo, showed reflow. In combination with heparin and aspirin, Ep alone (Group I) produced reflow at 42 ± 28 min and rt-PA alone (Group III) at 20 ± 17 min. The combination of Ep and only a single injection of rt-PA (Group II) significantly accelerated reflow to 5 ± 3 min (p = 0.002). In comparison to Group III dogs, combined therapy with Ep reduced the rt-PA dose required to initiate reflow. Statistical analysis comparing all experiments yielded significant overall differences with respect to patency status (p = 0.001). Coronary reocclusion was not observed in any dog given Ep, but occurred cyclically in four of six rt-PA-alone treated animals (p = 0.001).

Platelet aggregation and bleeding time. Enhancement of lysis was observed at doses of drug that inhibited platelet aggregation and prolonged bleeding time (Table 2). Complete abolition of ADP-induced platelet aggregation was observed in samples collected 10 min after the start of Ep infusion and persisted for the 90-min duration of treatment. Platelet aggregation did not change significantly from baseline values in rt-PA or placebo-treated dogs. Platelet counts and hematocrit measurements (data not shown) were within normal limits and remained unchanged from baseline values.

Pathology. Significant mural disruption was observed in all arteries. Arterial segments of persistently patent arteries in all animals revealed an irregular, denuded endothelium and areas of exposed media. Both the composition and amount of residual thrombus within the artery correlated with the type of treatment (Table 1). Animals treated with eptifibatide alone

Table 2. Platelet Aggregation and Bleeding Time

Group	Agent	Platelet Aggregation (%)*					Bleeding Time (min)			
		0 min	10 min	30 min	60 min	90 min	0 min	30 min	60 min	90 min
I	Ep	75 ± 16	0	0	0	0	1	11 ± 6	11 ± 6	9 ± 7
II	Ep + rt-PA	71 ± 24	0	0	0	0	2 ± 1	11 ± 5	11 ± 5	8 ± 6
III	rt-PA	65 ± 22	–	67 ± 31	71 ± 24	66 ± 23	1	3 ± 2	4 ± 4	2 ± 2
IV	saline	68 ± 11	–	63 ± 12	59 ± 19	69 ± 11	1	2 ± 1	1	1

*% light transmittance normalized to base line values. Values are mean ± SD.
Abbreviations as in Table 1.

(Group I) demonstrated residual erythrocyte-rich mural thrombus. The areas of exposed media and denuded endothelium were partially covered by platelets in an incomplete monolayer with no evidence of platelet clumping or aggregation. In contrast, animals treated with combination therapy (Group II) demonstrated a luminal surface that was predominantly clean, with a few scattered platelets and red blood cells, and minimal adherent mural thrombus without red cells or fibrin. Animals treated with rt-PA alone (Group III) contained platelet-rich mural thrombi. Finally, in placebo-treated animals (Group IV), the lumen was completely occluded with erythrocyte-rich thrombus.

Phase II

Observations after cessation of Ep infusion. First, coronary reflow was initiated in all dogs at 4 ± 1 min in the Ep + rt-PA group and at 44 ± 13 min in the Ep-alone group. Similar to the results from phase I, coronary artery patency was maintained throughout the 90-min infusion in all 10 dogs.

Experiments were then performed to determine the time required for restoration of platelet function following cessation of Ep infusion. Table 3 shows the time-dependent loss of Ep-induced inhibition of platelet aggregation: by 120 min, plasma levels of Ep had declined to <90 ± 34 ng/dl from the 944 ± 326 ng/dl achieved during infusion. The concentration of drug required to inhibit 50% (IC50) of ADP-induced platelet aggregation in dogs is 627 ± 63 nM using heparin anticoagulation. Thus, even at 90 ± 34 ng/ml (approximately 100 nM), there would be <10% inhibition of platelet aggregation. Table 3 also shows a time-dependent recovery of platelet function: by 120 min, platelet aggregation had returned to 86 ± 28% of normal from the complete inhibition achieved during infusion.

Pathology. To determine whether normally functioning platelets participated in thrombosis, experiments were performed to determine whether the arteries remained patent following cessation of the eptifibatide infusion. Although flow analysis revealed that all 10 arteries remained patent during the 120-min observation period, the SEM highlighted the differences between the two groups.

Arterial segments obtained from the 10 dogs at 120 min after the cessation of Ep when platelet function had returned nearly to baseline were analyzed by SEM. In dogs treated with Ep alone, a significant nonocclusive thrombus persisted at the site of thrombin injection. Additionally, there were newly adherent platelet aggregates on the residual thrombus and at multiple sites of endothelial disruption (Fig. 1). These SEM images are consistent with a thrombus in evolution where a retained, incompletely lysed, fibrin-rich, red-cell-rich thrombus functions as a nidus for the recruitment of activated platelets in the absence of GP IIb/IIIa receptor platelet blockade. In the dogs treated with Ep and rt-PA, SEM demonstrated an intimal surface with irregular, denuded endothelium and areas of exposed media. The luminal surface showed minimal intraluminal or mural thrombus without red cells or platelet adherence. Areas of exposed media showed either complete absence of cells of hematologic origin or scattered foci of platelet adherence (Fig. 2). Even in the setting of extensive intimal disruption with circumferential tears and high-grade stenosis, the injured de-endothelialized surface did not recruit new mural thrombus or new platelet aggregates at a time when platelet function was rapidly returning to baseline after cessation of the Ep infusion.

DISCUSSION

In the present study, we used a canine model of coronary thrombosis with endothelial disruption and superimposed distal stenosis to demonstrate that short-acting, reversible

Table 3. Platelet Aggregation and Eptifibatide Levels After Cessation of Eptifibatide Infusion

	Time Post Infusion (min)				
	0	30	60	90	120
Platelet aggregation (%)*	0	52 ± 10	48 ± 26	68 ± 29	86 ± 28
Plasma eptifibatide concentration (ng/ml)	944 ± 326	310 ± 182	215 ± 122	116 ± 45	<90 ± 34

*% light transmittance normalized to base line values. Values are mean ± SD

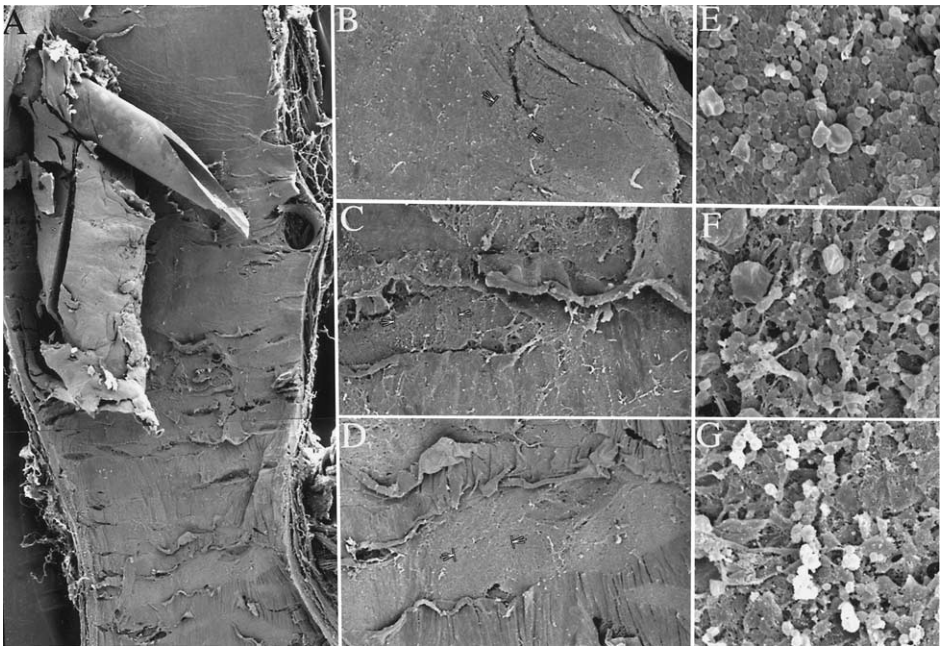

Figure 1. **A** is a low-power view of the longitudinally cut proximal left anterior descending coronary artery from a dog assigned to receive eptifibatide alone (in Phase II) showing presence of thrombus at the site of the side branch catheter through which thrombin was infused. Just distal to the thrombus, the traumatized left anterior descending coronary artery is seen. **B** and **E** are high-power views of the thrombus, showing platelet aggregation with fibrin strands and an occasional red cell. **(C** and **F** are high-power views of an area of the vessel severely damaged from crush injury, demonstrating disrupted endothelium covered by a thick layer of aggregated platelets within a fibrin mesh. **D** and **G** are high-power views from a more distal, less severely injury vessel segment showing a fibrin mesh with platelet adherence to the underlying collagen at the site of vessel injury.

GP IIb/IIIa inhibitor Ep, in combination with rt-PA, can acutely render a recanalized coronary artery resistant to platelet recruitment, new thrombus formation, and reocclusion despite the return of platelet function to normal. This model, with its exposed subendothelial structures, circumferential tears, residual mural thrombus, and high-grade

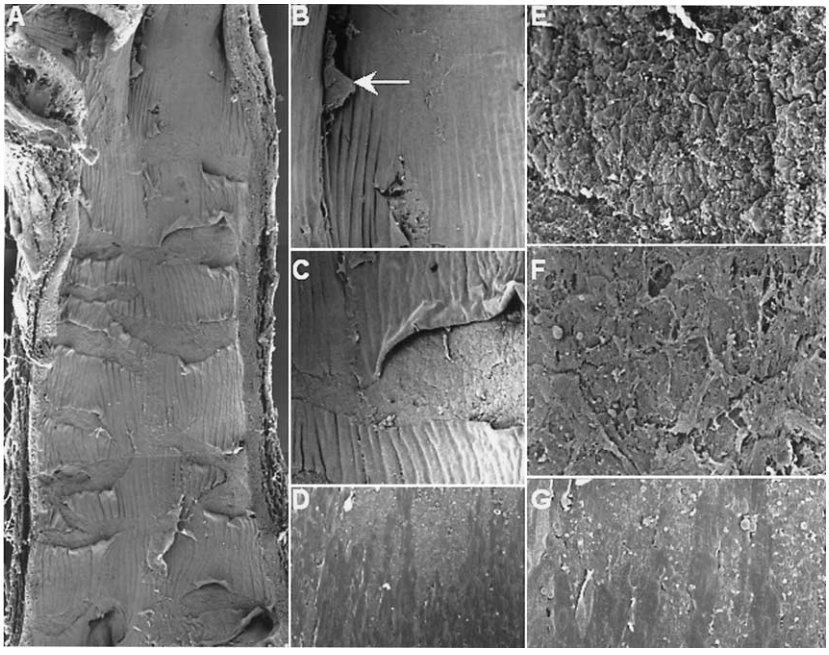

Figure 2. **A** is a low-power view of the longitudinally cut proximal left anterior descending coronary artery from a dog assigned to receive eptifibatide + rt-PA in Phase II. There is virtually no visible thrombus in marked contrast to that seen in Figure 1. **B** and **E** are high-power views of the small thrombus **(arrow)** showing a few scattered platelets adhering to the minimal thrombus. **C** and **F** are high-power views of an area of the vessel severely damaged from crush injury with absence of endothelium and exposure of the underlying collagen with only an occasional platelet adhering to the collagen and rare red cells. **D** and **G** are high-power views from a more distal area of the vessel with focal loss of endothelium, demonstrating only rare platelets adhering to the underlying surface. Compared to Figure 1, high-power views **F** and **G** demonstrate a marked reduction in the number of platelets adhering to two different segments of comparable vessel wall injury.

stenosis, constitutes a highly thrombogenic environment, thus representing a stringent challenge for the efficacy of a pharmacologic strategy to prevent recurrence of thrombus formation and acute reocclusion by neutralizing residual thrombogenicity at the site of endothelial disruption.

In the first phase of this study, we confirmed a principle previously established using 7E3 antibody and reduced dose rt-PA in our canine model of MI (14). During low-dose thrombolysis and high-level GP IIb/IIIa receptor blockade, SEM of the damaged arterial segment demonstrates a predominantly clean luminal surface, devoid of residual thrombus, with only select areas covered by scattered platelets.

In the absence of exogenous plasminogen activator, eptifibatide alone accelerated endogenous thrombolysis. However, SEM demonstrated erythrocyte-rich mural thrombus, indicative of thrombus incompletely lysed by the endogenous fibrinolytic system. Although reocclusion was prevented with a continuous Ep infusion, exogenous plasminogen activator was required for complete clearance of the coronary thrombus in this model.

Two hours after cessation of the eptifibatide infusion in the combination Ep + rt-PA group, SEM again demonstrated a predominantly clean surface, devoid of residual thrombus, with only a few areas covered with scattered platelets. Despite the return of normal platelet function, the subendothelial structures and circumferential tears in the damaged arterial segment failed to recruit new aggregates of platelets to the site of injury. This finding suggests that the damaged endothelial surface, even in the presence of high-grade stenosis, is no longer capable of recruiting and promoting platelet aggregation at the site of exposed subendothelial matrix.

In contrast, 2 h after cessation of Ep in the Ep-alone group, SEM demonstrated persistent residual erythrocyte-rich mural thrombus at sites of endothelial damage. At these sites of retained thrombus, new platelet aggregates and clumps were seen. Our findings demonstrate that high-level GP IIb/IIIa receptor blockade, combined with heparin and aspirin, is not sufficient to acutely neutralize the thrombogenicity of a damaged vessel wall in the presence of retained mural thrombus.

Multiple studies have established that the thrombogenicity of exposed subendothelial structures at the site of arterial wall damage is transient (17–20). Groves et al. (18) showed that after 8 h of platelet inhibition with prostaglandin, the damaged neointima in rabbits loses its ability to interact with platelets. Piepegras (19) showed that injured cat endothelium was nonreactive to platelets after 6 h of heparin. Wilentz et al. (20) subsequently demonstrated that most platelet deposition after deep balloon injury occurred within 30 min of endothelial disruption and returned to baseline within 4 h. Thus, if platelets are inhibited for a sufficient period of time following vessel wall injury, the injured wall loses its capacity to activate circulating platelets and its ability to form mural thrombus at the site of exposed subendothelium.

Residual mural thrombus alone, despite an ACT >250, can act as a potent stimulus for the recruitment of new platelets. Badimon et al. (21) have shown, in an isolated perfusion chamber, that formed thrombus is an even more potent stimulus to platelet recruitment than the exposed subendothelial structures. This intense thrombogenicity is primarily related to clot-bound thrombin (22) located in the interstices of the fibrin-rich thrombus (23,24), thereby protecting it from exposure to both heparin and antithrombin III.

Several studies have examined the effectiveness of GP IIb/IIIa platelet inhibition on prevention of thrombosis in canine models of arterial injury. In these models of coronary thrombosis induced by electrical injury (25,26) or segmental inversion (27,28), pretreatment of animals with 7E3 antibody consistently prevented coronary occlusion for 5 h following arterial damage during maximal GP IIb/IIIa receptor blockade. However, when animals were monitored beyond 5 h, reocclusion occurred in 22% to 60% of animals within one to six days (26,27). All arterial segments studied showed the presence of intraluminal macroscopic thrombus. Subsequently, Rote et al. (29) gave sequential rt-PA and 7E3 to dogs with electrically induced LAD thrombosis. During five days of follow-up, although all vessels remained patent, considerable macroscopic thrombus remained. In these studies, coronary patency was used as a surrogate marker for the recurrence of mural and intraluminal thrombus. Patency, however, does not indicate the absence of new thrombus formation. An artery may be patent, but, by continuing to recruit platelets on residual thrombus, may become susceptible to later reocclusion. Thus, patency in these studies is not a specific measure of the ability of the damaged arterial surface to recruit platelets. Scanning electron microscopy offers a more precise measure of the presence of thrombus, allowing direct visualization of the damaged endothelial surface and quantitation of fibrin and platelet aggregates. Our SEM-based demonstration of inhibition of platelet deposition at sites of injury establishes the principle that combination therapy acutely neutralizes the ability of the damaged endothelial surface to recruit new platelets by both inhibiting platelet aggregation and eliminating residual mural thrombus. This is the first study using an animal model of coronary artery thrombosis and SEM to demonstrate the ability of combination therapy to render a damaged endothelial surface resistant to new platelet deposition when platelet function has returned to normal.

This study is relevant to a continuing problem in the management of patients with ST elevation MI treated with thrombolytic therapy. In the early period after successful reflow, there is persistent instability of the culprit lesion because of residual high-grade stenosis and retained thrombus leading to reinfarction-recurrent ischemia. These complications were significantly reduced by combination therapy in GUSTO-V (30) and ASSENT-3 (31), although mortality was not significantly altered. Our findings do provide an additional biologic rationale for a pharmacologic strategy that not only

accelerates thrombolysis but also may provide short-term stability to a reperfused culprit lesion, permitting safe and deliberate transfer of patients to angioplasty-capable centers for definitive lesion stabilization.

STUDY LIMITATIONS

In the second phase of the study, our open-chest canine model of MI does not permit the long-term study of various reflow strategies. Nevertheless, the SEM at 2 h post cessation of Ep, when platelet function had returned to normal, reflects the state of the damaged endothelium in the absence of platelet inhibition. We do not know how the cellular biology of the damaged endothelium would have evolved had more time elapsed before sacrifice. Further studies will need to focus on the ability of this combination therapy to sustain long-term arterial patency.

CONCLUSIONS

Short-term, high level platelet GP IIb/IIIa receptor blockade combined with low-dose plasminogen activator is capable of reflowing and acutely rendering the injured and stenotic canine coronary artery resistant to platelet recruitment after return of normal platelet function. However, if thrombolysis is incomplete, the residual mural thrombus serves as a nidus for platelet reaccumulation. Thus, our study provides an additional rationale for the use of combined therapy to promote temporary stabilization of the culprit lesion, permitting transfer for percutaneous coronary intervention.

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REFERENCES

1. Davies MJ, Thomas AC. Plaque fissuring: the cause of acute myocardial infarction, sudden ischemic death, and crescendo angina. Br Heart J 1985;53:363–73.
2. Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death: autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion. Circulation 1985;71: 699–708.
3. Burke A, Farb A, Malcolm G, et al. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 1997;336:1276–82.
4. Friedman M. The coronary thrombus: its origin and fate. Hum Pathol 1971;2:81–128.
5. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242–50.
6. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (Part II). N Engl J Med 1992;326:310–8.
7. Adelman B, Stemerman MB, Mennel D, Handin RI. The interaction of platelets with aortic subendothelium: inhibition of adhesion and secretion by prostaglandin I2. Blood 1981;58:198–205.
8. Kaplan AV, Leung LLK, Leung WH, et al. Roles of thrombin and platelet membrane glycoprotein IIb/IIIa in platelet-subendothelial deposition after angioplasty in an ex vivo whole artery model. Circulation 1991;84:1279–88.
9. Badimon L, Badimon JJ, Lassila R, et al. Thrombin regulation of platelet interaction with damaged vessel wall and isolated collagen type I at arterial flow conditions in a porcine model: effects of hirudins, heparin, and calcium chelation. Blood 1991;78:423–34.
10. Yasuda T, Gold HK, Fallon JT, et al. A canine model of coronary artery thrombosis with superimposed high grade distal stenosis for the investigation of rethrombosis after thrombolysis. J Am Coll Cardiol 1989;13:1409–14.
11. Schaer DH, Ross AM, Wasserman AG. Reinfarction, recurrent angina, and reocclusion after thrombolytic therapy. Circulation 1988;76 Suppl II:II57–62.
12. Ohman EM, Califf RM, Topol EJ, et al. Consequences of reocclusion after successful reperfusion therapy in acute myocardial infarction. Circulation 1990;82:781–91.
13. Gold HK, Coller BS, Yasuda T, et al. Rapid and sustained coronary artery recanalization with combined bolus injection of recombinant tissue-type plasminogen activator and monoclonal antiplatelet GP IIb/IIIa antibody in a canine preparation. Circulation 1988;77: 670–7.
14. Yasuda T, Gold HK, Fallon JT, et al. Monoclonal antibody against the platelet glycoprotein (GP) IIb/IIIa receptor prevents coronary artery reocclusion after reperfusion with recombinant tissue-type plasminogen activator in dogs. J Clin Invest 1988;81:1284–91.
15. Lu HR, Gold HK, Wu Z, et al. Acceleration and persistence of recombinant tissue-type plasminogen activator induced arterial eversion graft recanalization with a single bolus injection of F(ab′)2 fragments of the antiplatelet glycoprotein IIb/IIIa antibody 7E3. Coron Art Dis 1991;2:1039–46.
16. Garabedian HD, Gold HK, Leinbach RC, et al. Bleeding time prolongation and bleeding during infusion of recombinant tissue-type plasminogen activator in dogs: potentiation by aspirin and reversal with aprotinin. J Am Coll Cardiol 1991;17:1213–22.
17. Groves HM, Kinlough-Rathbone RL, Richardson M, et al. Platelet interaction with damaged rabbit aorta. Lab Invest 1979;40:194–200.
18. Groves HM, Kinlough-Rathbone RL, Mustard JF. Development of nonthrombogenicity of injured rabbit aortas despite inhibition of platelet adherence. Atherosclerosis 1986;6:189–95.
19. Piepegras DG, Sundt TM, Didisheim P. Effect of anticoagulation and inhibition of platelet aggregation on thrombotic occlusion of endarterectomized cat carotid arteries. Stroke 1976;7:248–54.
20. Wilenz JR, Sanborn TA, Haudenschild CC, et al. Platelet accumulation in experimental angioplasty: time course and relation to vascular injury. Circulation 1987;75:636–42.
21. Badimon L, Chesebro JH, Badimon JJ. Thrombus formation on ruptured atherosclerotic plaques and rethrombosis on evolving thrombi. Circulation 1992;86 Suppl:III74–85.
22. Francis CW, Markham RE, Barlow GH, et al. Thrombin activity of fibrin thrombi and soluble plasmic derivatives. J Lab Clin Med 1983;102:220–30.
23. Weitz JI, Hudoba M, Massel D, et al. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but it is susceptible to inactivation by antithrombin III-dependent inhibitors. J Clin Invest 1990;86:385–91.
24. Hogg PJ, Jackson CM. Fibrin monomer protects thrombin from inactivation by heparin-antithrombin III: implications for heparin efficacy. Proc Natl Acad Sci USA 1989;86:3619–23.
25. Mickelson JK, Simpson PJ, Lucchesi BR. Antiplatelet monoclonal F(ab′)2 antibody directed against the platelet GP IIb/IIIa receptor complex prevents coronary artery thrombosis in the canine heart. J Mol Cell Cardiol 1989;21:393–405.
26. Bates ER, Walsh DG, Mu DX, et al. Passivation of the vessel wall-platelet interaction after deep coronary artery injury by temporary inhibition if the platelet glycoprotein IIb/IIIa receptor. Coronary Artery Dis 1992;3:67–76.
27. Kiss RG, Lu HR, Roskams T, et al. Time course of the effects of a single bolus injection of F(ab′)2 fragments of the antiplatelet GPIIb/IIIa antibody 7E3 on arterial eversion graft occlusion, platelet aggregation, and bleeding time in dogs. Arterioscler Thromb 1994;14:367–74.

28. Kiss RG, Stassen JM, Deckmyn H, et al. Contribution of platelets and the vessel wall to the antithrombotic effects of a single bolus injection of Fab fragments of the antiplatelet GPIIb/IIIa antibody 7E3 in a canine arterial eversion graft preparation. Arterioscler Thromb 1994; 14:375–380.
29. Rote WE, Mu DX, Bates ER, et al. Prevention of rethrombosis after coronary thrombolysis in a chronic canine model. I. Adjunctive therapy with monoclonal antibody 7E3 F(ab′)2 fragment. J Cardiovasc Pharmacol 1994;23:194–211.
30. GUSTO V Investigators. Reperfusion therapy for acute myocardial infarction with fibrinolytic therapy or combination reduced fibrinolytic therapy and platelet glycoprotein IIb/IIIa inhibition: the GUSTO V randomized trial. Lancet 2001;357:1905–14.
31. The Assessment of the Safety and Efficacy of a New Thrombolytic Regimen (ASSENT-3) Investigators. Efficacy and safety of tenecteplase in combination with enoxaparin, abciximab, or unfractionated heparin: the ASSENT-3 randomized trial in acute myocardial infarction. Lancet 2001;358:605–13.