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# Effect of time of 7E3 administration on rt-PA-induced reperfusion: study in a canine model of thrombus-based occlusion–reperfusion

Sam S. Rebello [a,*], Jinbao Huang [a], Kumi Saito [a], Jorge F. Saucedo [b], Eric R. Bates [b], Benedict R. Lucchesi [a]

[a] *Department of Pharmacology, University of Michigan Medical School, 1301C Medical Science Research Building III, Ann Arbor, MI 48109-0632, USA*
[b] *Department of Internal Medicine, University of Michigan Medical School, 13110 Taubman Center, Ann Arbor, MI 48109-0368, USA*

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**Abstract**

Chimeric version of the murine monoclonal antibody, 7E3 has been proposed for the early restoration of coronary artery patency during thrombolytic therapy. We determined the optimal time for administration of 7E3 during recombinant tissue plasminogen activator (rt-PA)-induced thrombolysis using a canine model of coronary artery thrombosis. After 30 min of thrombotic occlusion, microspheres were injected to assess regional myocardial blood flow, followed by a 90-min rt-PA infusion. Dogs were randomized to three groups wherein 7E3 (0.8 mg $kg^{-1}$, i.v.) was administered either 5 min before rt-PA (Group I), at the first evidence of thrombolysis (Group II), or after the completion of rt-PA infusion (Group III). Hemodynamic parameters were monitored for 6 h after which infarct size was estimated. Time to occlusion/reperfusion was similar in all groups. In the rt-PA alone group, 78% arteries reoccluded after 60 min of reperfusion. The incidence of reocclusion was lower in Groups II (25%, $P = 0.04$) and III (0%, $P < 0.01$). All arteries (100%) were patent at the end of the protocol in Group III vs 50% remaining patent in Group I ($P = 0.01$). Arterial patency was maintained longer in Group III (301 min, $n = 10$), compared with Groups I (124 min, $n = 5$) and II (124 min, $n = 6$). Arterial flow was greater in Group III (82%) compared with Groups I (27%) and II (35%) ($P < 0.01$). Regional myocardial blood flow and infarct size were similar in all groups. The data indicate that the time of administration of 7E3 in conjunction with rt-PA-induced thrombolysis influences patency status. The experimental results suggest that in the absence of aspirin and heparin, optimal thrombolysis is obtained when 7E3 is administered after the completion of rt-PA infusion regimen. 

*Keywords:* Thrombolysis; Reperfusion; Platelet; GPIIb/IIIa receptor antagonist; Infarct size

## 1. Introduction

Thrombolytic therapy has become the mainstay for treatment of acute myocardial infarction. With contemporary strategies for thrombolysis, approximately 50% of patients achieve grade 3 thrombolysis in myocardial infarction (TIMI 3) flow within 90 min of therapy. Since the residual thrombus in the infarct related artery continues to serve as a thrombogenic focus, almost 50% of these patients have recurrent episodes of myocardial ischemia and/or progress to reocclusion within hours to days. Therefore, only 25% of patients receiving thrombolytic therapy for myocardial infarction have optimal reperfusion (Moliterno and Topol, 1997). Reocclusion manifests as infarct extension and if untreated may lead to increased morbidity or mortality. Alternatively, thrombolysis involves generation of plasmin which is known to promote the synthesis of platelet activating factor (PAF) (Montrucchio et al., 1993a,b), activate complement proteins (Bennett et al., 1987) and enhance neutrophil aggregation (Montrucchio et al., 1996), all of which can have unwanted effects on arterial patency and myocardial function. Success of thrombolysis may also depend on the quality of flow in the reperfused infarct-related coronary artery. Frequent cyclic flow reductions during thrombolysis, indicative of platelet aggregation at the site of plaque rupture, can give rise to repeated, brief periods of ischemia–reperfusion and therefore, promote infarct extension. A non-oscillatory flow (TIMI grade 3), however, can limit the

* Corresponding author. Cardiovascular Biology, NW4, Rhône-Poulenc Rorer Research and Development, 500 Arcola Road, Collegeville, PA 19426-0107, USA. Tel.: +1-610-454-3275; Fax: +1-610-454-8740; E-mail: sam.rebello@rp-rorer.com

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extent of reperfusion injury and have a better outcome in terms of mortality and degree of ventricular dysfunction (Lincoff et al., 1995). In order to maintain arterial patency, agents such as GPIIb/IIIa receptor antagonists have been proposed to serve as adjuncts to thrombolysis. To fully assess the benefits of these agents, it is important to investigate the optimum time for their administration during thrombolysis.

In the first pilot small-scale trial, thrombolysis and angioplasty in myocardial infarction (TAMI 8), involving chimeric 7E3, bolus injections of ascending doses (0.1–0.25 mg $kg^{-1}$) were given at 3, 6, or 15 h after recombinant tissue plasminogen activator (rt-PA), and angiographic results indicated that 92% of treated patients had TIMI 2 and 3 flows as compared with 56% of control patients (Kleiman et al., 1993). Chimeric 7E3 was administered remotely with respect to rt-PA because of concern of serious bleeding. Two other phase II trials, one using integrilin (IMPACT-AMI) (Ohman et al., 1997) and the other using lamifiban (Moliterno et al., 1996) showed similar acute improvement in arterial patency. In these trials, integrilin and lamifiban were administered along with rt-PA. However, neither of these trials demonstrated clinical benefit in mortality at 30-day follow-up.

The goal of the present study was to examine the relative efficacy of adjunctive administration of 7E3 at various times during rt-PA-induced thrombolysis and to determine the optimal time for its administration using a canine model of coronary artery thrombosis and thrombolysis. To achieve this, 7E3 was administered before, during and after rt-PA-induced thrombolysis, and the corresponding effects on arterial patency, quality of flow and myocardial infarct size were assessed. The results indicate that optimal thrombolysis and maintenance of arterial patency can be achieved by administering 7E3 remotely to rt-PA.

## 2. Materials and methods

### 2.1. Materials

The murine monoclonal antibody, 7E3 $F(ab')_2$, was provided by Dr. Robert Jordan (Centocor, Malvern, PA). 7E3 $F(ab')_2$ was supplied as a sterile solution for injection (5 mg in 2.5 ml of 0.15 M NaCl and 0.01 M sodium phosphate, pH 7.2). Recombinant tissue plasminogen activator (rt-PA; Alteplase) was supplied by Genentech, (South San Francisco, CA). For all the studies described herein, 7E3 was administered intravenously as a bolus in 4–5 ml volumes. All other reagents used in this study were obtained from commercial sources.

### 2.2. Ethical considerations

The procedures followed in this study were in accordance with the guidelines of the University of Michigan (Ann Arbor) Committee on the Use and Care of Animals. Veterinary care was provided by the Unit for Laboratory Animal Medicine. The University of Michigan is accredited by the American Association of Accreditation of Laboratory Animal Care and the animal care and use program conforms to the standards in *The Guide for Care and Use of Laboratory Animals*, Department of Health, Education and Welfare publication no. NIH 78-23.

### 2.3. Model of coronary artery thrombolysis

Purpose-bred beagle dogs weighing 10–12 kg were anesthetized with sodium pentobarbital (30 mg $kg^{-1}$, i.v.), intubated and ventilated with room air (Harvard Apparatus, South Natick, MA). Catheters were inserted in the right and left femoral veins for the administration of rt-PA and 7E3, respectively. Blood pressure was recorded from the cannulated right femoral artery. The right carotid artery was cannulated for withdrawal of reference blood. The heart was exposed by a left thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. The left atrial appendage was cannulated for administration of radiolabeled microspheres used in the determination of regional myocardial blood flow. A 2-cm segment of the left circumflex coronary artery was isolated by blunt dissection. An ultrasonic flow probe (Model 1.5RB24, Transonic Systems, Ithaca, NY) was placed around the artery. An external stenosis was produced by securing a suture–ligature around the artery and an adjacent 18-gauge hypodermic needle and then removing the needle. An intracoronary electrode was fashioned from the tip of a 25-gauge (4 mm) hypodermic needle attached to a 30-gauge Teflon-insulated, silver-coated copper wire. The needle tip electrode was inserted through the arterial wall so that the uninsulated portion was positioned against the endothelial surface. External portion of the electrode was secured to the skin with a suture.

After a stabilization period of 60 min, anodal direct current was applied to the endothelial surface of the coronary artery via the previously implanted electrode. Electrolytic injury of the arterial wall was induced by connecting the intravascular electrode to the positive pole (anode) of a dual-channel square wave generator (Grass S88 stimulator and a Grass Constant Current Unit, Model CCU1A, Grass Instrument, Quincy, MA). The cathode was connected to a distant subcutaneous site. Current delivered to the artery was monitored continuously on an ammeter and maintained at 150 μA until persistent arterial occlusion was achieved. Under the conditions employed, thrombotic occlusion of the artery in the experimental model occurs within the first hour after the application of anodal current and induction of electrolytic injury to the intimal surface of the arterial wall. Standard limb lead II of electrocardiogram and coronary artery blood flow were recorded and monitored continuously to determine the time to occlusion. At the end of the protocol, all animals were euthanized by an overdose of anesthetic agent (pentobarbital sodium)

after which the heart was removed and processed for infarct size and regional myocardial blood flow determinations.

### 2.4. Determination of myocardial infarct size and area at risk

Histochemical determination of the anatomical area at risk and the zone of infarction was accomplished with a dual perfusion technique described previously (Mickelson et al., 1989; Black et al., 1995; Gralinski et al., 1996). The aorta was perfused in a retrograde fashion with 0.25% Evans Blue dye, while the left circumflex coronary artery was perfused with 100 ml of 1.2% triphenyltetrazolium chloride in 5 mM potassium phosphate buffer (pH 7.4, 37°C). The solutions were infused simultaneously for 5 min under a constant pressure of 100 mm Hg with the heart suspended in a water bath (37°C). The heart was cut into 1 cm thick transverse sections, weighed, and fixed in 10% buffered formalin. Both surfaces of each ventricular section were traced onto clear plastic overlays for subsequent quantification of the area at risk, denoted by the absence of Evans Blue dye, and the infarcted area, denoted by the absence of red formazan pigment within the area at risk. The tracings on the plastic overlays were digitized using a flat-bed scanner interfaced to a Macintosh computer (Apple Computer, Cupertino, CA). A MacDraft™ (Innovative Data Design) software program was used to calculate the respective areas of each of the demarcated regions and their respective weights from the digitized images. The infarct size was expressed as a percentage of the area at risk and as a percentage of the total left ventricle.

### 2.5. Determination of regional myocardial blood flow

Regional myocardial blood flow was determined using radiolabeled microspheres (15 μm diameter; New England Nuclear, Boston, MA) by the reference withdrawal method. Each vial of microspheres was placed in an ultrasonic bath and subsequently vortexed before injection to insure adequate dispersion of the microspheres before administration. Microsphere (1 ml of 100 μCi $^{103}$Ru) injection was performed using the left atrial appendage after achieving 30 min of persistent coronary artery occlusion. Reference arterial blood samples were obtained simultaneously from the right femoral and carotid arteries at a constant rate (3.5 ml $min^{-1}$) using a withdrawal pump, beginning 30 seconds before microsphere injection and ending 60 s later. The reference sample counts were averaged for calculation of myocardial blood flow. Tissue samples weighing 0.10–0.50 g (wet weight) were dissected from the posterior papillary muscle, endocardial, mid-myocardial, and epicardial sections of the heart from the regions of distribution of the left circumflex artery (ischemic zone) and left anterior descending artery (non-ischemic zone) of the left ventricle. Four sections from each heart were used so that blood flow to each region represented the average of four samples for each animal. The mean regional myocardial blood flows from the inner two-thirds of the myocardium were used to determine whether an excessive collateral blood supply ($> 0.18$ ml $min^{-1}$ (g tissue)$^{-1}$) was present at 30 min of thrombotic occlusion of the coronary artery.

### 2.6. Platelet studies and coagulation measurements

Whole blood (20 ml) was withdrawn from the left femoral vein for assessing hematologic parameters. Blood was collected in plastic syringes containing 3.7% sodium citrate as the anticoagulant (1:10 citrate/blood vol/vol). Platelet count was determined with an H-10 cell counter (Texas International Laboratories, Houston, TX). Platelet-rich plasma, the supernatant present after centrifugation of anticoagulated whole blood at $140 \times g$ for 10 min, was used for aggregation studies. Platelet-poor plasma was prepared after the platelet-rich plasma was removed by centrifuging the remaining blood at $2000 \times g$ for 10 min and discarding the bottom cellular layer. Ex vivo platelet aggregation was assessed with a four-channel aggregometer (BioData-PAP-4, Bio Data, Hatboro, PA) by recording the increase in light transmission through a stirred suspension of platelet-rich plasma (adjusted to $200 \times 10^3$ platelets $\mu l^{-1}$) maintained at 37°C. Platelet aggregation was induced with adenosine diphosphate (ADP; 20 μM) and γthrombin (70 nM). A subaggregatory dose of epinephrine (550 nM) was used to prime the platelets before the agonists were introduced. Values are expressed as percent aggregation, which are represented by the fraction of light transmission standardized to platelet-poor plasma samples yielding 100% light transmission.

To assess the anticoagulation state of the animals, the activated partial thromboplastin time and prothrombin time were determined using a Hemochron™ (Technidyne, Edison, NJ) with reagents supplied by the manufacturer. Citrated whole blood was used for these determinations.

### 2.7. Experimental protocol

The experimental protocol for coronary artery thrombolysis and infarct size determination is outlined in Fig. 1. Upon stabilization from the surgical interventions, blood was drawn from the left femoral vein for baseline hematological tests. Anodal current was applied to the coronary artery. After thrombotic occlusion (denoted as $T = 0$) a 30 min period was provided for clot aging. At this point, the anodal current application was discontinued and radiolabeled microspheres were administered. This was followed immediately by initiation of the 90 min rt-PA infusion. Time to reperfusion was defined as the time from rt-PA administration to the first evidence of reperfusion. Time to reocclusion was defined as the time from reperfusion to complete cessation of blood flow. The artery was defined

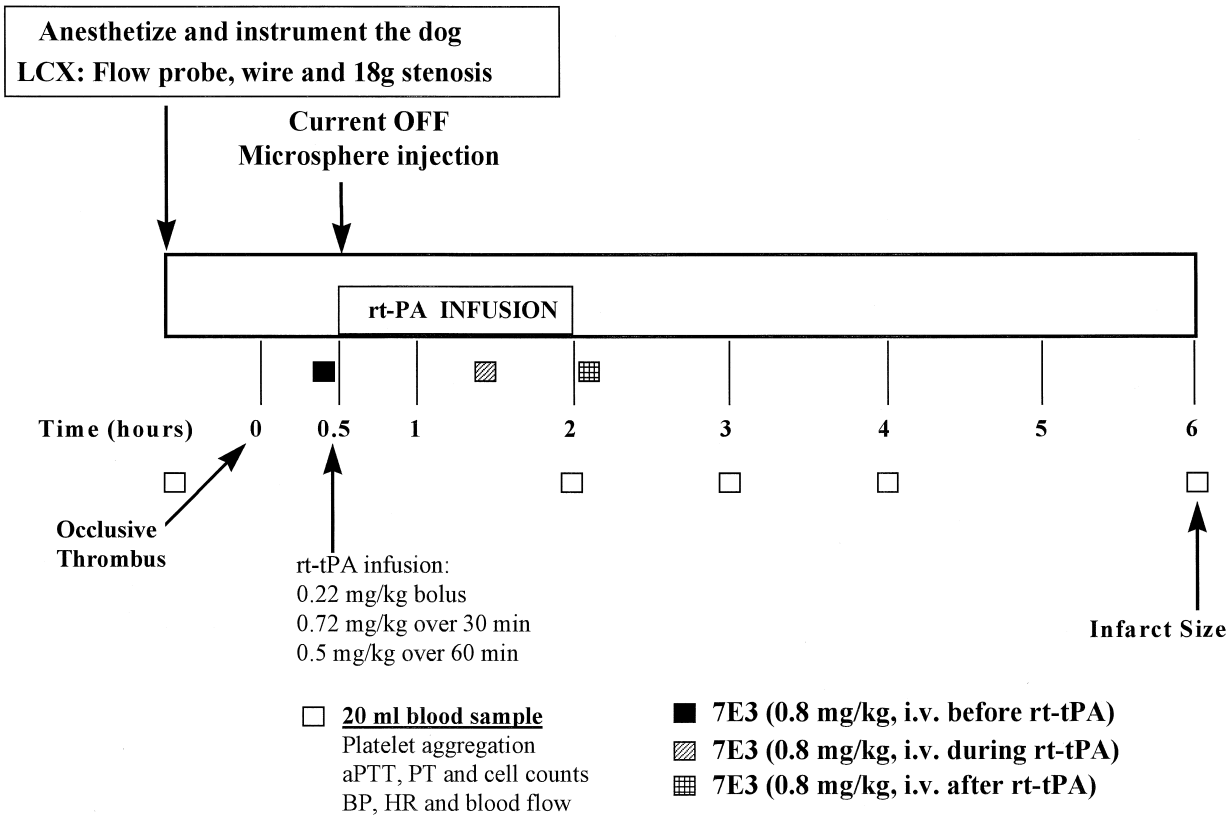


Fig. 1. Representation of the experimental protocol to investigate the effect of time of 7E3 administration in a canine model of coronary artery thrombolysis. Animals were anesthetized, instrumented and stabilized before induction of thrombosis. Thirty minutes after stable occlusion, radiolabeled microspheres were administered to determine regional myocardial flow. This was followed immediately by rt-PA infusion to achieve thrombolysis. 7E3 (0.8 mg/kg, i.v.) was administered at various stages of thrombolysis. Hemodynamic parameters and blood flow were monitored for 6 h. Periodic blood samples were drawn to assess coagulation parameters. LCX = left circumflex coronary artery; aPTT = activated partial thromboplastin time; PT = prothrombin time.

as patent when the measured blood flow was $>1$ ml $min^{-1}$ for $>1$ min. Dogs receiving rt-PA were randomized to receive 7E3 (0.8 mg $kg^{-1}$, i.v.) either 5 min before rt-PA (7E3 before rt-PA group), at the first sign of thrombolysis (7E3 during rt-PA group), or after the completion of the 90 min infusion (7E3 after rt-PA group). Periodic blood samples were collected to assess the effect of 7E3 on coagulation parameters. Mean arterial pressure, heart rate and blood flow were monitored continuously for 6 h. At the end of the protocol, animals were euthanized by an anesthetic overdose, and the hearts were excised to estimate infarct size and to determine regional myocardial blood flow.

### 2.8. Exclusion criteria

Predetermined exclusion criteria were (1) the presence of heart worms upon final post-mortem examination of the heart; (2) failure to manifest electrocardiographic evidence of myocardial ischemia (failure to demonstrate sustained ST segment elevation) in lead II of the electrocardiogram and discoloration (cyanosis) of the epicardial surface in the region of distribution of the left circumflex coronary artery after its occlusion; (3) regional myocardial blood flow greater than 0.18 ml $min^{-1}$ (g tissue)$^{-1}$ in the inner two thirds of ventricular wall at risk of infarction; (4) refractory ventricular fibrillation requiring more than three attempts of cardioversion using low energy (10 J) pulses applied directly to the surface of the heart; (5) a circulating platelet count of less than 100,000 $\mu l^{-1}$; (6) inability for epinephrine-primed platelets to aggregate in response to ADP (20 $\mu$M) and $\gamma$thrombin (70 nM) at baseline; and (7) thrombotic occlusion of the coronary artery more than 3 h from the onset of arterial wall injury.

### 2.9. Statistical analysis

The data are expressed as mean $\pm$ S.E.M. The relationship between infarct size and regional myocardial blood flow was assessed by analysis of covariance (ANCOVA). Data obtained for the hemodynamics, hematological and occlusion parameters were subjected to one-way analysis of variance (ANOVA) (repeated measures) followed by Fisher's protected least significant difference (PLSD) as a post-hoc test to determine significance at $P < 0.05$. Platelet

aggregation data were subjected to a paired *t*-test to assess the differences over time within a group, and values were determined to be statistically different at a level of $P < 0.05$. The incidence of reperfusion, reocclusion and mortality between groups was compared using Fisher's Exact Test.

## 3. Results

### 3.1. Group characteristics

A total of 64 dogs were entered in the study. The mean body weight of the dogs in all the groups ranged from 10–12 kg. Three dogs exhibited intractable ventricular fibrillation immediately after coronary artery occlusion. All but 11 (Control, no reperfusion) of the remaining 61 animals received rt-PA to achieve thrombolysis. Dogs administered rt-PA were further divided into four groups to receive saline ($n = 12$), or 7E3 either before rt-PA (7E3 before rt-PA, $n = 14$), at the first sign of rt-PA-induced reperfusion (7E3 during rt-PA, $n = 10$) or after the completion of rt-PA infusion (7E3 after rt-PA, $n = 14$). The systemic hemodynamic parameters were similar in all the groups at baseline. However, there was a decrease in mean arterial blood pressure and an increase in heart rate which was common to all the groups during rt-PA-induced reperfusion. For example, the mean arterial blood pressure (heart rate) values at baseline were $102 \pm 5$ ($159 \pm 7$), $96 \pm 4$ ($164 \pm 7$), $105 \pm 4$ ($169 \pm 8$) and $88 \pm 9$ ($166 \pm 7$) mmHg (beats $min^{-1}$) in rt-PA, 7E3 before rt-PA, 7E3 during rt-PA and 7E3 after rt-PA groups, respectively. At

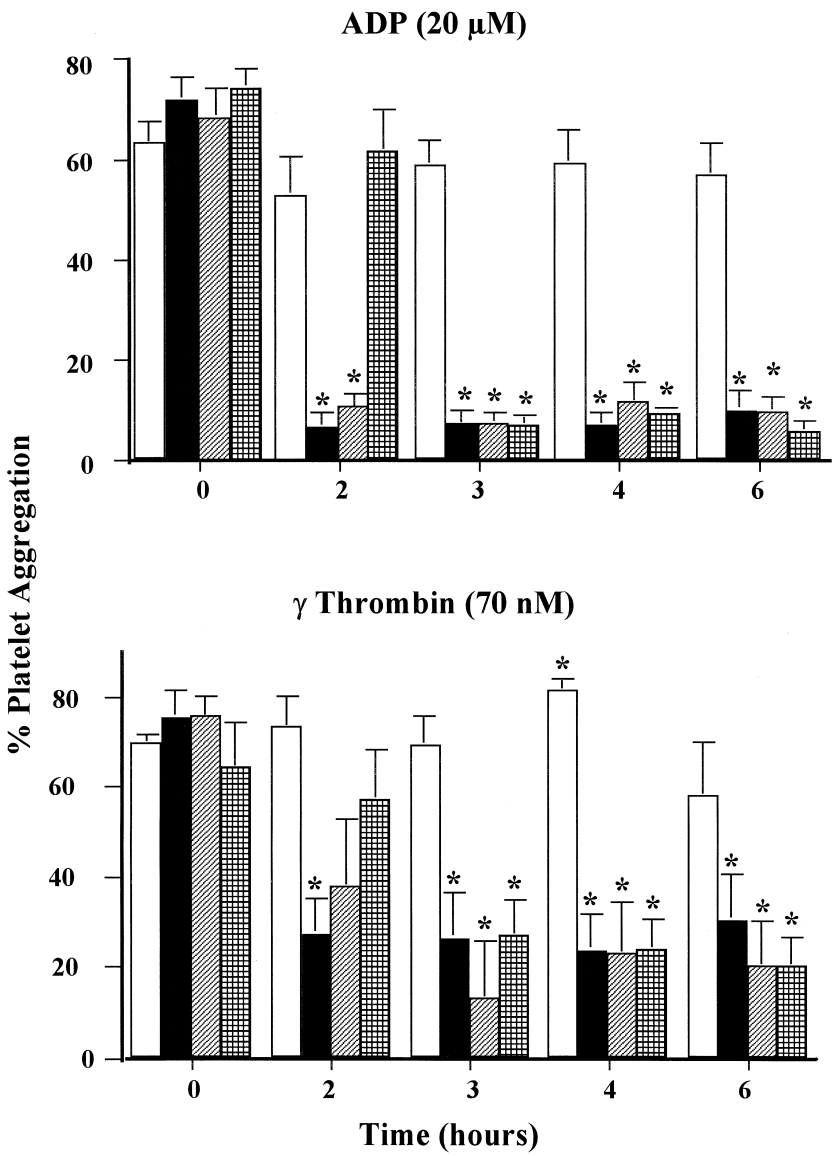


Fig. 2. Effect of 7E3 administration before (filled columns), during (hatched columns) and after (grided columns) rt-PA-induced thrombolysis on ex vivo platelet aggregation induced by ADP (20 μM) and γthrombin (70 nM). Open columns represent rt-PA alone group. Values are expressed as mean ± S.E.M. Preselected time intervals represent time after occlusive thrombus formation. $^{*}P < 0.05$ compared to baseline.

the end of the protocol ($T = 6$ h) the mean arterial blood pressure (heart rate) values in these groups were $73 \pm 6$ ($174 \pm 6$), $66 \pm 4$ ($193 \pm 5$), $67 \pm 4$ ($184 \pm 11$) and $73 \pm 3$ ($194 \pm 15$) mmHg (beats $min^{-1}$). One-way ANOVA (repeated measures) indicated that all the rt-PA groups were statistically similar with regards to mean arterial blood pressure ($P = 0.24$) and heart rate ($P = 0.60$) changes.

### 3.2. Ex vivo coagulation parameters

At baseline, the plasma activated partial thromboplastin time and prothrombin time were 23.9 s and 13.5 s, respectively. Administration of rt-PA alone or in combination with 7E3 did not alter the activated partial thromboplastin time and prothrombin time in any group. ADP and γthrombin-induced platelet aggregations were studied using citrated blood samples collected at preselected time points as shown in the protocol. At baseline, the ADP-induced percent platelet aggregation values were $63 \pm 4$, $72 \pm 4$, $68 \pm 6$ and $74 \pm 11$ in rt-PA, 7E3 before rt-PA, 7E3 during rt-PA and 7E3 after rt-PA groups, respectively ($P > 0.05$) (Fig. 2). Depending upon the time of administration of 7E3, the platelet aggregation response was inhibited significantly during the protocol. The maximum inhibition produced by 7E3 in each of the three groups was 89–95% ($P < 0.0001$). Likewise, γthrombin-induced platelet aggregation values at baseline were $69 \pm 2$, $75 \pm 6$, $75 \pm 4$ and $64 \pm 10$ in rt-PA, 7E3 before rt-PA, 7E3 during rt-PA and 7E3 after rt-PA groups, respectively ($P > 0.05$) (Fig. 2). The maximum inhibition produced by 7E3 in all three groups ranged from 68–82% ($P = 0.004$). Infusion of rt-PA alone did not affect the ex vivo aggregation status significantly.

### 3.3. Occlusion parameters and mortality

Electrolytic injury to the left circumflex coronary artery produced typical cyclic flow reductions which led to a progressive decrease in blood flow culminating in an occlusive thrombus formation in approximately 43 min. Infusion of rt-PA, 30 min after persistent occlusion, resulted in lysis of the thrombus in approximately 42 min. Therefore, the total time for which the coronary artery remained occluded was ~72 min. The times to occlusion ($P = 0.52$) and reperfusion ($P = 0.30$) were found to be similar for all the groups (Table 1).

In the rt-PA group, 7/9 (78%) arteries reoccluded after 60 min of reperfusion. Single administration of 7E3 at different time points during rt-PA-induced reperfusion had a varying effect on the incidence of reocclusion at the end of the protocol and the total time for which the arteries remained patent. The incidence of reocclusion was significantly lower only when 7E3 was administered either during (2/8, 25%, $P = 0.04$), or after (0/10, 0%, $P = 0.0007$) rt-PA infusion. The 7E3 before rt-PA group (5/10, 50%) was similar ($P = 0.18$) to the rt-PA group in terms of incidence of reocclusion at the end of the protocol. All arteries (100%) were patent at the end of the protocol when 7E3 was administered after rt-PA infusion versus 50% remaining patent when 7E3 was administered 5 min before rt-PA ($P = 0.01$). The effect of time of 7E3 administration also was evident on the time to reocclusion which was significantly greater in the 7E3 after rt-PA group as compared to the 7E3 before rt-PA and 7E3 during rt-PA groups. The quality of flow in the recanalized arteries was determined on the basis of number of cyclic flow reductions observed throughout the protocol (Table 1). Most of the cyclic flow reductions (mean = $11 \pm 1$) occurred during the infusion of rt-PA, and therefore the 7E3 after rt-PA and rt-PA groups were similar. However, when the number of cyclic flow reductions that occurred after 7E3 administration were counted there were almost no cyclic flow reductions in the 7E3 after rt-PA group as compared to those in the 7E3 before rt-PA and 7E3 during rt-PA groups.

During the protocol, rt-PA-induced reperfusion was associated with reperfusion arrhythmia which, in some instances culminated in ventricular fibrillation. The incidence of mortality did not differ among the four treatment groups (Table 1).

### 3.4. Coronary artery blood flow and patency status

Before the induction of electrolytic injury, the coronary artery blood flow was $8.1 \pm 0.5$, $8.0 \pm 0.9$, $7.9 \pm 1.3$, $9.0 \pm 0.9$ and $8.0 \pm 0.5$ ml $min^{-1}$ in the control, rt-PA, 7E3

Table 1
Occlusion parameters and mortality

| Groups | Time to occlusion (min) | Incidence and time to reperfusion | | Incidence and time to reocclusion | | Number of CFRs | | Mortality, *n* (%) |
|---|---|---|---|---|---|---|---|---|
| | | *n* (%) | (min) | *n* (%) | (min) | Total | After 7E3 | |
| Control ($n = 11$) | $38 \pm 9$ | – | – | – | – | 0 | – | 0/11(0) |
| rt-PA ($n = 12$) | $43 \pm 7$ | 11/12 (92) | $48 \pm 7$ | 7/9 (78) | $60 \pm 5$ | $11 \pm 1$ | – | 2/12 (17) |
| 7E3 before rt-PA ($n = 14$) | $37 \pm 7$ | 12/14 (86) | $39 \pm 7$ | 5/10 (50) | $124 \pm 32$[a] | $8 \pm 2$ | $8 \pm 2$ | 2/14 (14) |
| 7E3 during rt-PA ($n = 10$) | $55 \pm 10$ | 10/10 (100) | $47 \pm 8$ | 2/8 (25)[a] | $124 \pm 4$[a] | $7 \pm 1$ | $6 \pm 1$ | 2/10 (20) |
| 7E3 after rt-PA ($n = 14$) | $44 \pm 7$ | 14/14 (100) | $33 \pm 5$ | 0/10 (0)[a,b] | $301 \pm 5$[a,b,c,d] | $13 \pm 2$[b,c] | $1 \pm 0$[b,c] | 4/14 (28) |

CFRs = Cyclic flow reductions.
[a] $P < 0.05$ compared to rt-PA; [b] $P < 0.05$ compared to 7E3 before rt-PA; [c] $P < 0.05$ compared to 7E3 during rt-PA.
[d] Since all the arteries were patent, the maximum duration of reperfusion was used arbitrarily for statistical analysis.

before rt-PA, 7E3 during rt-PA and 7E3 after rt-PA, respectively ($P = 0.93$) (Fig. 3). Infusion of rt-PA, 30 min after persistent occlusion, resulted in thrombolysis in ~42 min in all the groups. In the rt-PA group, however, the flow declined progressively after cessation of the 90 min infusion. Administration of 7E3 provided evidence for maintenance of coronary artery blood flow in the recanalized artery. However, the duration of arterial patency and/or the quality of blood flow was dependent upon the sequence of administration of 7E3 relative to the administration of rt-PA. When 7E3 was administered during or after rt-PA infusion there was significantly greater blood flow when compared to rt-PA alone. Blood flow in rt-PA alone group was similar to that in the 7E3 before rt-PA group ($P > 0.05$). Within the 7E3 groups, significantly greater blood flow was observed in the 7E3 after rt-PA group as compared to the 7E3 before rt-PA and 7E3 during rt-PA groups. At the end of the protocol ($T = 6$ h), the mean blood flow in 7E3 after rt-PA group was 82% as compared to only 27% in 7E3 before rt-PA group ($P < 0.0001$) and 35% in 7E3 before rt-PA group ($P = 0.003$).

The effect of timing of 7E3 administration relative to that of rt-PA on the patency status of coronary artery is depicted in Fig. 4. The arteries were defined as patent if the blood flow was $> 1$ ml min$^{-1}$ for $> 1$ min. In the rt-PA alone group, the arterial flow oscillated frequently between the occluded and patent state, and the majority of the arteries reoccluded before completion of the protocol. Administration of 7E3 after the completion of rt-PA infusion had a favorable effect in terms of maintaining most of the recanalized arteries in a patent state and minimizing the number of cyclic flow reductions.

### 3.5. Regional myocardial blood flow and infarct size

Blood flow in endocardial, mid-myocardial and epicardial regions of the left circumflex coronary artery and left anterior descending coronary artery was determined by the microsphere technique 30 min after persistent thrombotic occlusion of the coronary artery (Table 2). The collateral blood flow in regions supplied by the left circumflex coronary artery did not differ among groups ($P = 0.51$). Myocardial blood flow in the regions supplied by the left anterior descending artery ranged from 0.548–0.877 ml min$^{-1}$ g$^{-1}$ and was similar in all the groups ($P = 0.33$). The relationship between infarct size and collateral blood flow for individual dogs was examined in order to control for differences in the extent of myocardial injury due to variation in collateral blood flow. One factor ANCOVA was used wherein the treatment was a factor in the model, inner two-thirds collateral flow in the left circumflex region (comprising of the papillary, endocardial and mid-myocardial regions) is a regressor (independent variable), and the infarct size (expressed as percent of the area at

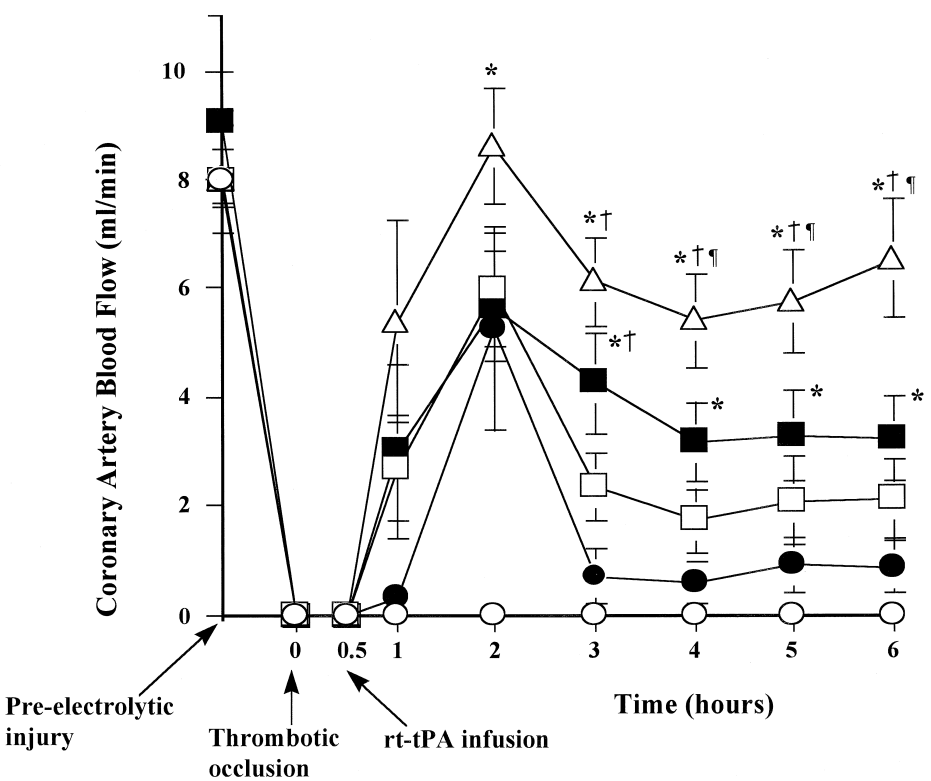


Fig. 3. Effect of 7E3 administration before (open squares), during (filled squares) and after (open triangles) rt-PA-induced thrombolysis on coronary artery blood flow. *$P < 0.05$ compared with rt-PA alone group (filled circles). †$P < 0.05$ compared with 7E3 before rt-PA. ¶$P < 0.05$ compared with 7E3 during rt-PA. Open circles represent control group of animals that did not receive rt-PA. Values are expressed as mean ± S.E.M.

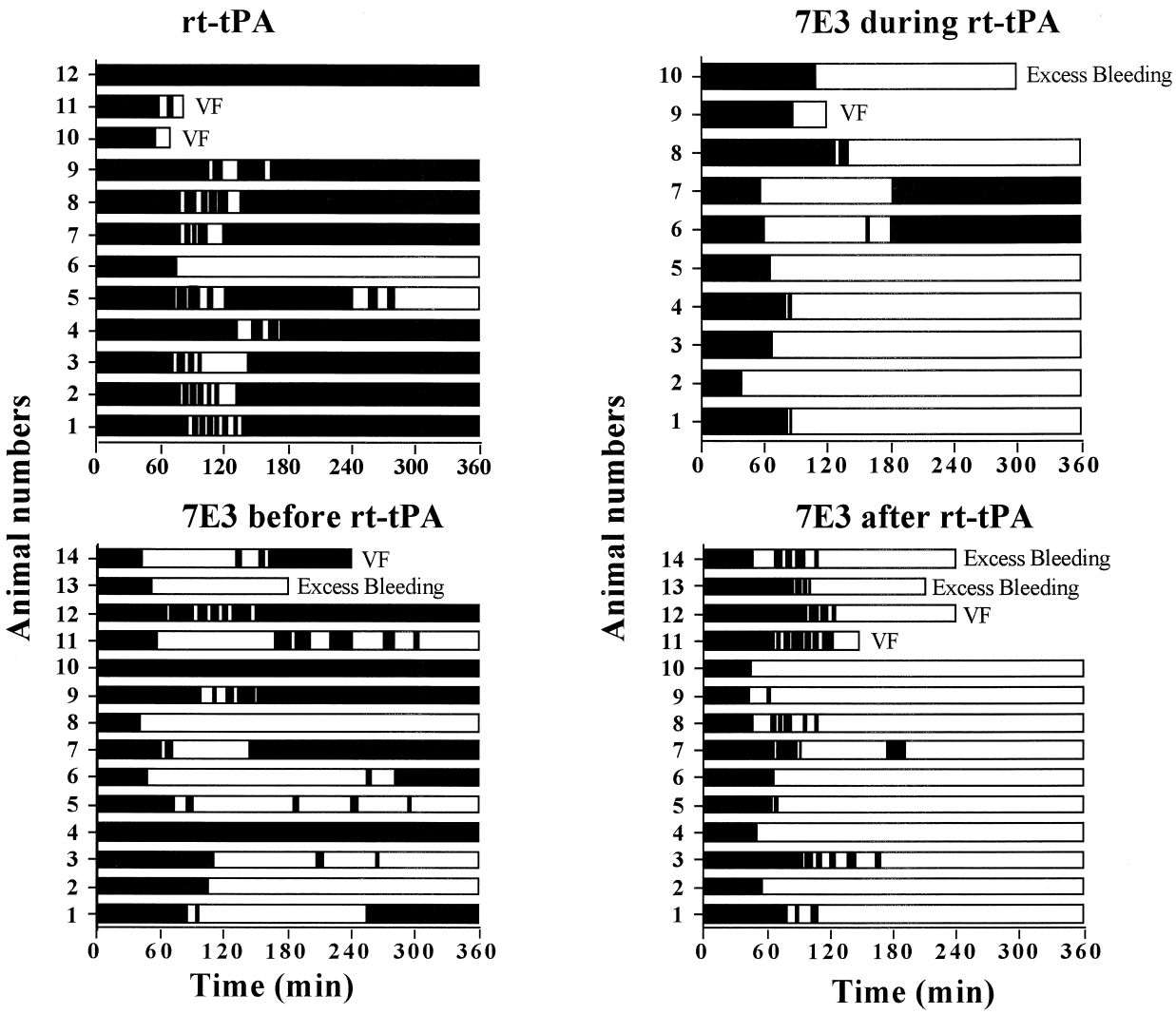


Fig. 4. Effect of time of 7E3 administration during the thrombolysis protocol. Each horizontal bar indicates data from an individual animal in a group. Open box represents patent artery and closed box represents occlusion.

risk) served as the dependent variable. Because the *F*-value for various treatments was not significant ($P = 0.11$), it was concluded that the infarct size was similar for all the animals with different treatments. However, a *P* value of 0.03 for the collateral blood flow effect indicated that the latter was a predictor of infarct size, and therefore served a purpose in the analysis by controlling for differences which existed prior to the experiment.

Table 2
Regional myocardial blood flow

| Groups | LCX flow (ml $min^{-1}$ $g^{-1}$) | | | LAD Flow (ml $min^{-1}$ $g^{-1}$) | | |
|---|---|---|---|---|---|---|
| | ENDO | MID | EPI | ENDO | MID | EPI |
| Control ($n = 11$) | 0.088 ± 0.039 | 0.077 ± 0.026 | 0.131 ± 0.039 | 0.622 ± 0.058 | 0.618 ± 0.061 | 0.611 ± 0.073 |
| rt-PA ($n = 12$) | 0.067 ± 0.013 | 0.076 ± 0.016 | 0.107 ± 0.023 | 0.647 ± 0.061 | 0.646 ± 0.07 | 0.688 ± 0.088 |
| 7E3 before rt-PA ($n = 14$) | 0.072 ± 0.02 | 0.081 ± 0.025 | 0.147 ± 0.051 | 0.709 ± 0.121 | 0.647 ± 0.102 | 0.548 ± 0.087 |
| 7E3 during rt-PA ($n = 10$) | 0.082 ± 0.028 | 0.087 ± 0.029 | 0.129 ± 0.044 | 0.877 ± 0.101 | 0.847 ± 0.108 | 0.802 ± 0.089 |
| 7E3 after rt-PA ($n = 14$) | 0.076 ± 0.02 | 0.103 ± 0.014 | 0.161 ± 0.025 | 0.723 ± 0.061 | 0.674 ± 0.065 | 0.714 ± 0.10 |

LCX, left circumflex coronary artery;
LAD, left anterior descending coronary artery.

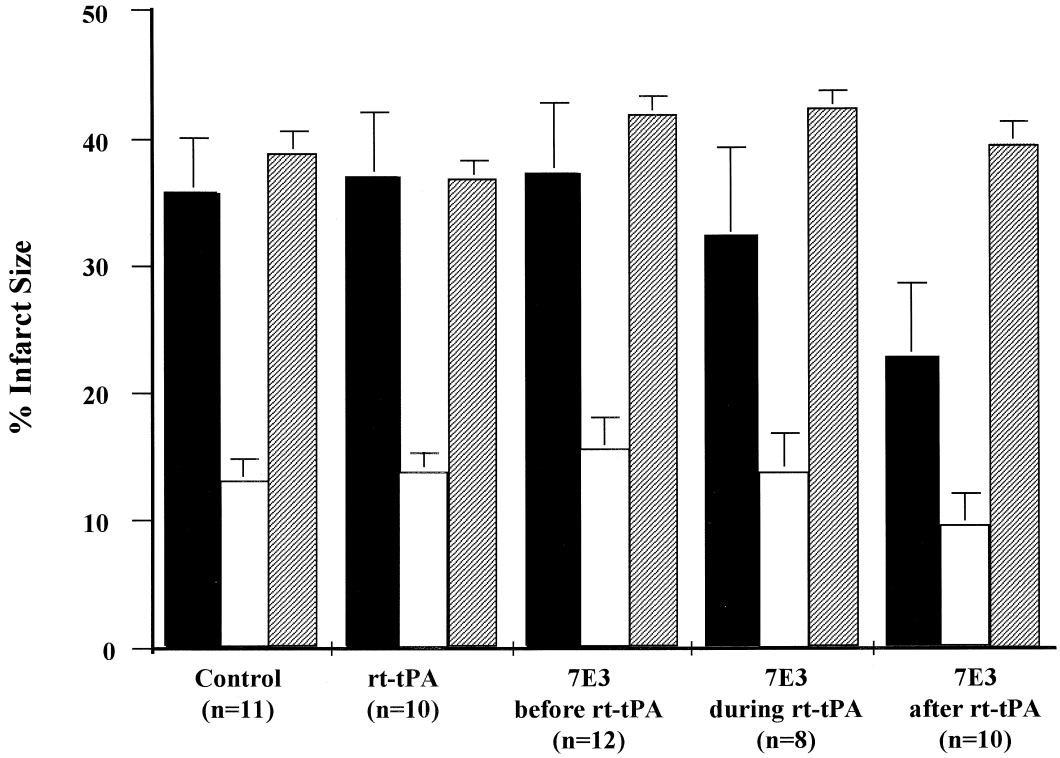


Fig. 5. Effect of 7E3 administration on myocardial infarct size, expressed as a percentage of the area at risk (filled columns) or percentage of the left ventricle (open columns). Hatched columns represent area at risk as the percentage of left ventricle. Values are expressed as mean ± S.E.M.

The mean infarct size data is shown in Fig. 5. The area at risk as a percentage of left ventricle was similar in all the groups. The infarct size as a percentage of total left ventricle and area at risk showed a trend to be low in the 7E3 after rt-PA group as compared with the 7E3 before rt-PA group; however, the change failed to reach statistical significance ($P = 0.06$).

## 4. Discussion

The open artery hypothesis states that early and rapid reperfusion through the infarct-related coronary artery leads to a better clinical outcome than that observed in the absence of reperfusion. Reopening an occluded coronary artery results in the salvage of ischemic myocardium, limitation of infarct size, preservation of left ventricular function and reduction in mortality (White and Braunwald, 1998). The reduction in mortality that can be achieved with thrombolytic therapy in patients with an evolving myocardial infarction depends on the elapsed time between onset of symptoms and initiation of treatment, or more specifically, on the duration of coronary occlusion before reperfusion (Vermeer et al., 1986). The beneficial effect of thrombolytic therapy is substantially better in patients presenting within 2 h after symptom onset compared to those presenting later (Boersma et al., 1996). Lytic therapy has become a widely accepted treatment approach for acute myocardial infarction. However, with current thrombolytic therapy, only 25% of patients have optimal thrombolysis (Moliterno and Topol, 1997). Due to its ability to block the platelet GPIIb/IIIa receptors, chimeric 7E3 (abciximab, ReoPro™) may represent a promising adjunct to thrombolysis. Preclinical studies using a variety of animal models have shown that 7E3 when administered in conjunction with rt-PA prevents abrupt reocclusion of the recanalized coronary artery (Gold et al., 1988, Yasuda et al., 1988; Fitzgerald et al., 1989; Mickelson et al., 1990; Rote et al., 1994). However, the optimum time to administer the antiplatelet agent relative to the administration of rt-PA has not been examined in a systematic manner.

We used a canine model wherein electrolytic injury-induced thrombus was first formed and later lysed by rt-PA. Recanalization of the coronary artery was followed by 80% incidence of reocclusion. Antagonism of GPIIb/IIIa receptors by 7E3 was associated with maintenance of coronary blood flow for 6 h. However, the latter effect was dependent on the time of administration of 7E3. For example, administration of 7E3 5 min before rt-PA infusion resulted in a 50% incidence of reocclusion. A significant effect was observed when 7E3 administration was postponed until the completion of rt-PA infusion. In this group, none of the animals progressed to reocclusion and the amount of flow (82% of preocclusion value) in the injured arteries was significantly higher than in other groups under study. When 7E3 was given at the first sign

of thrombolysis an intermediate effect was observed in terms of incidence of reocclusion (25%) and maintenance of flow. The results obtained in the 7E3 after rt-PA group are in agreement with those reported earlier using the same model and similar mode of administration of 7E3 (Mickelson et al., 1990; Rote et al., 1994). However, the results obtained in the 7E3 before rt-PA contrast with several earlier reports (Gold et al., 1988, Yasuda et al., 1988; Fitzgerald et al., 1989) which used different experimental models. Fitzgerald et al. (1989) showed that coadministration of 7E3 with rt-PA shortened the time to reperfusion and prevented reocclusion in a long-term sedated canine model in which coronary artery occlusion was induced by electrolytic injury (200 μA) in the absence of a critical stenosis. The total dose of rt-PA given to each animal varied (10 $\mu g\ kg^{-1}\ min^{-1}$ until 10 min after lysis) and rt-PA was not administered until 2 h after thrombosis. Studies in a canine model with localized thrombin-induced clot in the externally traumatized left anterior descending coronary artery with a critical stenosis showed that coadministration of 7E3 with rt-PA facilitated initial thrombolysis and prevented rethrombosis (Gold et al., 1988; Yasuda et al., 1988). This model relies on the conversion of fibrinogen to fibrin by thrombin, and the resulting thrombus is erythrocyte- and fibrin-rich (Yasuda et al., 1993). In the latter studies the total dose of rt-PA given to each animal varied [0.225 or 0.45 mg ($kg^{-1}$) bolus] every 15 min up to four doses (Gold et al., 1988) or 15–30 $\mu g\ kg^{-1}\ min^{-1}$ for 30–60 min (Yasuda et al., 1988), and other agents (heparin, lidocaine and procainamide) were administered concomitantly.

It may appear logical to administer 7E3 early on during thrombolysis because various clinical trials have shown that early restoration of coronary patency improves survival (Vermeer et al., 1986; Boersma et al., 1996). Also, since thrombolytic therapy with rt-PA gives rise to plasmin-mediated platelet activation (Fitzgerald et al., 1989; Montrucchio et al., 1993a,b), it appears to be judicious clinically to administer 7E3 along with rt-PA to negate the participation of platelets in acute rethrombosis. The present study, however, calls attention to the fact that the number of cyclic flow reductions were greater when 7E3 was given early as compared to those when 7E3 was given remotely to rt-PA. The cyclic variations in arterial flow lead to a progressive reduction in blood flow as a result of local accumulation of platelets and narrowing of the arterial lumen. As the aggregates increase in size over several minutes, coronary artery flow declines, and a pressure gradient across the platelet mass develops, leading to sudden dislodgment of the thrombus and an abrupt increase in flow. Because cyclic flow reductions appear to be reliable markers of the local formation of platelet aggregates, this phenomenon can be predictive of immediate complications during thrombolysis. The pathophysiology of cyclic flow reductions, with a potential for progression to acute myocardial infarction or sudden lethal arrhythmia, closely resembles the sequence that occurs in humans (Ikeda et al., 1993). Therefore, administration of 7E3 after complete thrombolysis may promote a non-oscillatory flow pattern and increase the success of thrombolysis. The latter is evident from Fig. 3 which demonstrates improved and longer perfusion for 7E3 administered after thrombolytic therapy. The coronary flow in this group was already 50% higher at the end of the 90 min rt-PA infusion. Delay in 7E3 administration may have allowed rt-PA to achieve more and complete lysis of the thrombus thereby resulting in better restoration of flow, an observation underscoring the effect of time of 7E3 administration during thrombolysis.

The reduced efficacy of early 7E3 administration in the present experimental model is difficult to explain. During the period of thrombotic occlusion, the local plasmin activity at the site of the thrombus would become maximal. Platelets bind plasminogen at physiologic zymogen concentrations and this interaction may serve to localize and promote plasminogen activation. In the presence of rt-PA, platelet bound plasminogen would contribute to plasmin generation at the site of the thrombus (Miles and Plow, 1985). Thus, platelets have the potential to contribute to fibrinolysis by providing a site for localizing plasminogen within a thrombus and by influencing plasmin formation. It may be speculated therefore, that the local proteolytic actions of plasmin upon 7E3 may have reduced the efficacy of the monoclonal antibody. This interaction would be less likely to occur in the other groups wherein 7E3 was administered after thrombolysis was achieved. Under the latter circumstances the local plasmin activity in and about the residual thrombus would decrease due to the restoration of blood flow. An additional explanation for the observed reduction in efficacy of rt-PA administered in the presence of platelet bound 7E3 may be found in the study of Miles et al. (1986). It was noted that unstimulated and stimulated thrombasthenic platelets, deficient in GPIIb/IIIa receptors, had a markedly reduced capacity to bind plasminogen. Therefore, it remains possible that the previous administration of the monoclonal GPIIb/IIIa receptor antibody would result in a environment in which platelets assume a pseudo-thrombasthenic state thus having a reduced capacity to contribute to plasmin generation at the site of the thrombus.

Administration of 7E3 after thrombolytic therapy demonstrated improved and longer perfusion, but there was no difference in myocardial injury. Unlike a clinical study, the number of animals in the present study groups ranged from 10 to 14. Therefore, the study was not powered enough to detect significance in a limited duration (6 hours) of the protocol. The times to occlusion and reperfusion were statistically similar, but small changes may have added to the variability and hampered any changes in the final infarct size. Although the number of cyclic flow reductions were higher in the rt-PA groups compared to none in the control group, there was no

increase in infarct size indicating that cyclic flow reductions did not influence infarct extension in the present short-term model. However, the overall infarct size in the control group (~35% percent of risk area) appears to be less as compared to that reported in manual occlusion–reperfusion studies using similar periods ischemia/reperfusion and assessment of area at risk (Mickelson et al., 1989; Black et al., 1995; Gralinski et al., 1996). Unlike that in the manual occlusion–reperfusion models, initial ischemia in the thrombosis–thrombolysis model is slow and progressive, and often preceded by two to three cyclic flow reductions. In view of the concept of myocardial preconditioning (Murray et al., 1986; Murray et al., 1990), the intermittent episodes of ischemia–reperfusion imposed by cyclic flow reductions may have allowed for washing out of toxic metabolites, and provided some degree of myocardial protection. It is also possible that sudden relief of a manual occlusion versus gradual reperfusion during thrombolysis may have influenced the ensuing amount of reperfusion injury.

It may be important to highlight some limitations of the present study. Unlike routine clinical practice, we did not use heparin and aspirin because it may have introduced an additional variable therefore confounding the interpretation of data. However, this may be one likely reason for the discrepancy between our results and those from clinical studies demonstrating thrombolytic efficacy of abciximab in combination with reteplase (SPEED Investigators, 1998), or rt-PA (TIMI 14 Investigators, 1998). Also, the time to treatment (rt-PA administration) was fixed (30 minutes after thrombotic occlusion) unlike the clinical situation where the time varies. Finally, due to the limited duration of the protocol, it is possible that we may have missed the long-term effect of reduction in cyclic flow on infarct size by 7E3 administered after rt-PA.

## 5. Conclusion

In conclusion the results suggest that 7E3 administration in a canine model of thrombus based occlusion–reperfusion is associated with maintenance of arterial patency during rt-PA-induced thrombolysis. In the absence of heparin and aspirin, the maximum adjunctive benefit of 7E3 was observed by administering the platelet GPIIb/IIIa receptor antibody after achieving thrombolysis and restoration of blood flow in the injured coronary artery.

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