Increased Thromboxane Biosynthesis During Coronary Thrombolysis

Evidence That Platelet Activation and Thromboxane A₂ Modulate the Response to Tissue-Type Plasminogen Activator In Vivo

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Platelet activation is markedly increased during coronary thrombolysis and limits the response to thrombolytic therapy. A possible mediator of platelet activation in this setting is thromboxane (TX) A₂, a potent platelet agonist formed in greatly increased amounts during coronary thrombolysis in man. To address this hypothesis, we examined the role of TXA₂ in modulating the response to intravenous tissue-type plasminogen activator (t-PA) in a chronic canine model of coronary thrombosis. Reperfusion occurred in 60±5 minutes and was complicated by spontaneous reocclusion. The times to reperfusion and reocclusion were platelet-dependent. Consistent with a role for TXA₂ in this process, TXA₂ biosynthesis, determined as excretion of its enzymatic metabolite, 2,3-dinor-TXB₂, was markedly increased during coronary thrombolysis. Furthermore, inhibition of TXA₂ by aspirin, given alone or in combination with a TXA₂/prostaglandin endoperoxide receptor antagonist, accelerated reperfusion and partly inhibited cyclic flow variations during reperfusion. The delay in reperfusion and reocclusion induced by TXA₂ appeared to be mediated by platelet aggregation since the F(ab')₂ fragment of 7E3, a monoclonal antibody to the platelet GPIIb/IIa, also accelerated reperfusion and prevented reocclusion without altering TXA₂ biosynthesis. These findings suggest that platelet aggregation limits the response to coronary thrombolysis and that platelet activation in this setting is partly TXA₂-dependent. (Circulation Research 1989;65:83-94)

Thrombolytic therapy of acute myocardial infarction is limited by failure to reperfuse the occluded artery in 20–50% of patients and by acute reocclusion in 20–30% of those who initially reperfuse. In addition, experimental studies suggest that myocardial injury, a major determinant of survival, increases rapidly over the first few hours of coronary occlusion, whereas reperfusion may be delayed an hour or more following intravenous administration of a thrombolytic agent. The mechanisms of delayed or failed reperfusion in man are unknown, but studies in experimental models suggest a role for platelets. Platelets are a major component of coronary thrombi in man, particularly at their point of attachment to the vessel wall. In addition, recent studies demonstrate marked platelet activation during coronary thrombolysis with intravenous streptokinase and tissue-type plasminogen activator (t-PA). Thus, the high density of platelet aggregates in human coronary thrombi and continued activation of platelets during coronary thrombolysis may limit the efficacy of thrombolytic agents.

What mediates platelet activation during coronary thrombolysis is unknown. However, we have recently demonstrated a marked increase in thromboxane (TX) A₂ biosynthesis in patients with acute myocardial infarction treated with either intravenous streptokinase or t-PA. The major cyclooxygenase product of arachidonic acid in platelets, is a potent platelet activator and induces platelet aggregation in response to a variety of platelet agonists, including ADP, epinephrine, and low concentrations of thrombin and collagen. In the pres-
ence of TXA2 receptor antagonists, these agents fail to induce complete, irreversible aggregation despite platelet activation and formation of TXA2. TXA2 biosynthesis is increased11 and studies with the TXA2 inhibitor, aspirin, imply a pathogenic role for TXA2 in a number of platelet-dependent conditions, including acute myocardial infarction,12 unstable angina,13 and pregnancy-induced hypertension.14 These observations are consistent with the hypothesis that TXA2 is an important regulator of platelet function in vivo. Formation of this eicosanoid during coronary thrombosis8,9 exceeds that seen in unstable angina and acute myocardial infarction,15 suggesting that it may also exert a biological effect in this setting. In these studies, we examine the functional significance of the increased TXA2 formation that occurs during coronary thrombosis with t-PA in a chronic canine model of coronary thrombosis.

Materials and Methods

ADP and arachidonic acid were purchased from Sigma Chemical, St. Louis, Missouri, and U46619 from The Upjohn Co, Kalamazoo, Michigan. We gratefully acknowledge gifts of t-PA (GR11044, Dr. Elliot Grossbard, Genentech, South San Francisco, California), 7E3 and OC125 monoclonal antibodies (Dr. Barry Coller, SUNY, New York, New York, and Dr. Robert E. Jordan, Centocor, Malvern, Pennsylvania), epoprostenol (Dr. R. Kent, Burroughs Wellcome Foundation, Research Triangle Park, North Carolina), L636,499 (Dr. Anthony Ford-Hutchinson, Merck-Frosst, Pointe Claire–Dorval, Quebec, Canada), and AH23848 (Dr. Patrick Humphrey, Glaxo, Ware, Hertfordshire, UK).

Experimental Model

The experimental protocol was approved by the Animal Care Committee at Vanderbilt University. Coronary thrombosis was induced by electrical injury to the endothelium, as previously described.15,16 Male mongrel dogs (17–23 kg) were anesthetized with pentobarbital 30 mg/kg i.v. and ventilated using a respirator (Harvard Apparatus, The chest was closed, and the animal was allowed to recover. Heparin sulfate (2,000 units) was administered subcutaneously every 8 hours for 48 hours. Preliminary studies demonstrated that with this anticoagulant regimen no clot formed at the electrode site even at 10 days after surgery.17

Experimental Procedure

Five to 7 days after surgery, the animal was sedated with morphine (1–2 mg/kg) and acepromazine (1–2 mg/kg) (total dose given over the period of study). This regimen had no effect on blood pressure or ex vivo platelet aggregation. The electrode was connected in series with a potentiometer, an ammeter, and the anode of a 9-volt battery, and the circuit was completed by grounding to the subcutaneous tissues. The ultrasonic flow probe was connected to a directional pulsed Doppler flowmeter for continuous recording of coronary blood flow. Blood pressure was recorded in a subset of animals through a catheter placed in the femoral artery by the Seldinger technique and connected to a strain gauge manometer. All tracings, including an electrocardiogram, were recorded onto a strip chart using a physiological recorder (model 79D, Grass Instruments, Quincy, Massachusetts). Coronary thrombosis was induced by passing a 200-μA current through the electrode and resulted in complete coronary occlusion in 1–2 hours. Two hours after complete occlusion, defined as the absence of coronary flow detected by the flow probe, t-PA 10 μg/kg/min was infused until 10 minutes after complete reperfusion had been achieved. This was defined as a coronary blood flow greater than or equal to that at baseline. After reperfusion, the animal was observed for 4 hours.

In four experiments, coronary angiography was performed through the femoral artery at intervals during the experiment using a 7F Amplatz catheter (Cordis, Miami, Florida). Coronary angiograms were obtained in the left anterior oblique projection after the injection of 3–4 ml of contrast using a Philips image intensifier (Atlanta, Georgia).

Urine was collected by catherization before surgery and at intervals on the day of study for determination of 2,3-dinor-TXB2 and 2,3-dinor-6-keto-prostaglandin (PG) F1α, major enzymatic metabolites of TX and prostacyclin (PGI2), respectively.18,19 These studies were not performed in experiments where the femoral artery was cannulated since the resulting trauma artifically elevates eicosanoid biosynthesis. Peripheral venous blood was obtained through a 19-gauge cannula for platelet aggregation, coagulation, and hematological studies at intervals throughout the experiment.

Pharmacological Studies

PGI1 (epoprostenol) was dissolved in glycine buffer, pH 10.5, immediately before use. Active drug or vehicle was administered intravenously beginning either 30 minutes before infusion of t-PA or after reperfusion and was continued for 1 hour after reperfusion. Ex vivo platelet aggregation was determined immediately before and 30 minutes after the initiation of the PGI1 infusion and again after withdrawal of PGI1.

To examine the role of TXA2 in this model, we examined the effect of aspirin and of two structur-
ally distinct TXA2/prostaglandin endoperoxide receptor antagonists: 3-carboxyl-dibenzo (b,f) theipin-5,5-dioxide (L636,499)\textsuperscript{20} and [1(Z),2EI,5]-7-[[(1,1-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclo pentyl]-4-heptenoic acid (AH23848).\textsuperscript{21} Aspirin was dissolved in 0.1 M Na2CO₃ immediately before use and was administered intravenously over 10 minutes in a dose of 20 mg/kg either 30 minutes before t-PA administration or after reperfusion. L636,499 was administered in 0.05 M Na2CO₃ at a dose of 20 mg/kg over 10 minutes followed by an intravenous infusion of 2 mg/kg/min. AH23848 was administered in 0.1 M NaHCO₃ in a single dose of 1 mg/kg given i.v. over 10 minutes either 30 minutes before t-PA administration or after reperfusion.

In a separate series of studies, we examined the response to a monoclonal antibody (7E3) to the platelet glycoprotein Ib/IIa complex,\textsuperscript{22,23} the putative fibrinogen receptor. The F(ab')\textsubscript{2} fragment of 7E3 was compared with the F(ab')\textsubscript{2} fragment of OC125, a monoclonal antibody to a human ovarian cell carcinoma antigen, which has no antiplatelet activity.\textsuperscript{5} The antibodies were administered in a dose of 0.8 mg/kg given over 10 minutes through a peripheral vein commencing 30 minutes before the administration of t-PA. At this dose, 83% of platelet binding sites for 7E3 are occupied.\textsuperscript{6} Platelet aggregation was determined before and 30 minutes after antibody administration, after reperfusion, and again at 4 hours after reperfusion. The platelet count in whole blood was determined before administration of the antibody and again at the completion of the experiment.

Platelet Aggregation

Platelet aggregation studies were performed in platelet-rich plasma (PRP) by light transmission,\textsuperscript{24} using a multichannel aggregometer (Biodata PAP-4, Biodata, Hartboro, Pennsylvania). PRP was prepared by centrifuging citrated venous blood (3.8% Na citrate, 9:1 vol/vol) at 3,000 rpm for 50 seconds. This allowed us to examine the response to PG1\textsubscript{2}, which is rapidly inactivated in biological fluids. Platelet-poor plasma was obtained by centrifuging the remaining blood at 3,000 rpm for 10 minutes. Platelet aggregations were performed in 500 µl aliquots with aggregating agents added in volumes of 10% or less. For all agonists, a threshold concentration, that is, the minimum concentration inducing maximum and irreversible aggregation, was identified and used to assess the response to interventions. The concentration of aggregating agents is expressed as the final concentration in PRP. Platelet aggregation was expressed as percent light transmission, where PRP equals 100%. In vitro, canine platelets will fully aggregate in response to the TXA2/prostaglandin endoperoxide analogue, U46619, but must first be partially activated by epinephrine or ADP. Less frequently, canine platelets require priming before they will respond to arachidonic acid. Therefore, to examine the response to U46619 and arachidonic acid, the platelets were first primed with ADP at a concentration (1–2 µM) that induced a small (<10%), reversible wave of aggregation, as previously described.\textsuperscript{25}

Biochemical Studies

Urinary 2,3-dinor TXB\textsubscript{2} and 2,3-dinor-6-keto PGF\textsubscript{1α} were determined by gas chromatography, negative ion-chemical ionization, and mass spectrometry using their respective tetradeuterated analogues as internal standards. Briefly, to a 5-ml sample of urine was added 5 ng of each internal standard and the urine extracted by immunoaffinity chromatography as previously described.\textsuperscript{26} After formation of the methoxime derivatives, further purification was achieved by thin layer chromatography and the sample derivatized to the trimethylsilyl ether. Final separation and quantification was achieved by a gas chromatograph in series with a Nermag R10–10 mass spectrometer operated in the negative ion mode. Serum TXB\textsubscript{2} was determined by radioimmunoassay following incubation of whole blood in a glass test tube at 37°C for 45 minutes.\textsuperscript{27}

Coagulation and Hematological Studies

Fibrinogen concentration was determined before and 2 hours after t-PA administration as thrombin-clottable protein in citrated plasma using a fibrometer (BBL, Becton Dickinson and Co, Cockeysville, Maryland). The partial thromboplastin time (PTT) was determined as the time to clot formation after the addition of activated rabbit brain cephalin (Actin, American Dade, Aguada, Puerto Rico) to citrated plasma. The platelet count in citrated whole blood was determined using a Coulter counter. All samples were analyzed immediately to minimize artifacts due to ex vivo fibrinogenolysis and were repeated 2 hours after discontinuation of the infusion of t-PA.

Statistical Analysis

Groups were compared by Wilcoxon rank-sum test or by the Kruskal-Wallis one-way analysis of variance where appropriate. These are nonparametric tests and, therefore, are independent of the distribution of the data.\textsuperscript{28}

Results

Control Experiments

In control experiments, t-PA 10 µg/kg/min induced reperfusion in all animals, with a time to reperfusion ranging from 42 to 80 minutes (mean 60±4.5) (n=9). In contrast, administration of vehicle (0.9% sodium chloride) had no effect, and spontaneous reperfusion did not occur over 4 hours of
FIGURE 1. Continuous recording of coronary blood flow velocity in the circumflex coronary artery in a single experiment. In the upper panel coronary thrombosis is induced and results in complete coronary occlusion. Two hours later, reperfusion is induced by tissue-type plasminogen activator (tPA). Reperfusion is complicated by episodic coronary occlusion and reperfusion, which is not prevented by heparin.

observation (n=5). Reperfusion was complicated in all cases by episodes of gradual reocclusion followed by abrupt reperfusion (Figure 1) and, ultimately, by complete coronary occlusion at 32±5.2 minutes (range 16–49). Angiographic studies in four animals demonstrated that, following reperfusion, there was a severe coronary stenosis (80–90%), which was the site of subsequent reocclusion (Figure 2). In additional experiments, histological studies demonstrated that the coronary narrowing reflected residual thrombus that, at higher magnification and on electron microscopy, was found to be rich in platelets (Figure 3).

Biochemical Studies
Urinary excretion of 2,3-dinor-TXB₂ (range 494–1,819, median 779 pg/mg creatinine) and 2,3-dinor-6-keto PGF₁α (range 129–554, median 244 pg/mg creatinine) was not significantly different on the day of study compared with presurgical levels. After induction of coronary thrombosis, excretion of both metabolites increased and remained elevated throughout the 2 hours of occlusion (Figure 4). After reperfusion with t-PA, there was a further marked increase in urinary excretion of 2,3-dinor-TXB₂ (peak 998–5,067, median 3,356 pg/mg creatinine) and 2,3-dinor-6-keto-PGF₁α (peak 203–1,399, median 1,004 pg/mg creatinine). No further increase was seen in animals in whom coronary thrombosis was induced and treated with vehicle only (Figure 4) or after infusion of t-PA 10 µg/kg/min for 60 minutes in animals in whom coronary thrombosis was not induced (3,113±1,043 versus 5,212±1,132 pg/mg creatinine after t-PA infusion, n=3). Administration of aspirin 20 mg/kg i.v. 30 minutes before reperfusion with t-PA abolished the increase in both metabolites (Figure 5).

Platelet and Coagulation Studies
Fibrinogen concentration in plasma decreased during the infusion of t-PA and was similarly depressed (range 10–23%, median 11%) 2 hours after completion of the infusion. However, plasma fibrinogen also decreased in vehicle-treated controls to a similar extent (range 4–23%, median 11%). Platelet aggregation to the threshold concentration of ADP decreased slightly during and following the infusion of t-PA (65±7.0% versus 80±3.0%, p<0.05) whereas aggregation to arachidonic acid and U46619 was unaltered.

Pharmacological Studies
Studies with PGI₂. In six experiments, reocclusion occurred despite the administration of heparin (200 units/kg i.v.) and marked prolongation of the PTT at the time of reocclusion (77±32 versus 12 seconds, p<0.05) (Figures 1 and 6). Reocclusion also occurred in the absence of heparin. In contrast, PGI₂ (500 ng/kg/min) prevented reocclusion and stabilized coronary blood flow in 10 of 11 animals (Figure 6). This occurred whether PGI₂ was administered before reperfusion (n=6) or during cyclic coronary flow following reperfusion (n=5). Discontinuation of the PGI₂ resulted in abrupt reocclusion in all cases (Figure 6) with a mean time to reocclusion of 14±3 minutes (range 8–29 minutes). At the doses used, PGI₂ markedly depressed ex vivo platelet aggregation to ADP (15±4.6% versus 69±7.4%;
FIGURE 2. Serial angiograms of the circumflex coronary artery during a single experiment. Following induction of coronary thrombosis complete coronary occlusion occurred. Two hours later, tissue-type plasminogen activator was administered in a dose of 10 μg/kg/min, resulting in reperfusion 1 hour thereafter. Reocclusion occurred rapidly at the site of residual narrowing with the distal vessel now filling through a collateral from the left anterior descending coronary artery.

Studies with TXA2 inhibitors. Administration of aspirin reduced serum TXB2 by 96±5%, abolished the platelet aggregation response to arachidonic acid (data not shown), and decreased urinary 2,3-dinor-TXB2 by 60±8%. Aspirin prevented reocclusion and stabilized coronary flow in some experiments (Table 1, Figure 7, upper panel) while in others it had no effect (Figure 7, lower panel). In part, this may have reflected incomplete inhibition of TXA2 biosynthesis since urinary 2,3-dinor-TXB2 was still markedly elevated in some experiments early following aspirin administration (Figures 5 and 7). Furthermore, addition of a TXA2/prostaglandin endoperoxide-receptor antagonist, L636,499, enhanced the response to aspirin without inducing a further reduction in 2,3-dinor-TXB2 (Figure 7). The reverse was also true; that is, addition of aspirin enhanced the response to L636,499. A similar effect was seen with a structurally distinct TXA2/prostaglandin endoperoxide-receptor antagonist, AH23848 (Table 1). The effect of these treatments on reocclusion was similar whether they were administered before t-PA or following reperfusion. Pre-treatment of animals with aspirin also decreased the time to reperfusion (range 23–60, median 42 minutes) although the effect was more marked when aspirin was combined with the TXA2/prostaglandin endoperoxide-receptor antagonist, AH23848 (range 15–36, median 25 minutes) (Table 1).

Studies with the F(ab')2 fragment of 7E3. Administration of the 7E3 monoclonal antibody to the platelet glycoprotein IIb/IIIa abolished platelet aggregation to ADP, U46619, and arachidonic acid (Figure 8) over the 4 hours of observation but had no effect on blood pressure or heart rate. 7E3 also shortened the time to reperfusion, abolished cyclical flow variations after reperfusion, and prevented reocclusion in all experiments (Figure 9, Table 2). In contrast, the control antibody, OC125, had no effect on ex vivo platelet aggregation. Furthermore, OC125 did not alter the time to reperfusion with t-PA, and in all experiments reperfusion was complicated by cyclic coronary blood flow, and ultimately, complete reocclusion occurred. There was no signifi-
FIGURE 3. Histological sections through the circumflex coronary artery after reperfusion with tissue-type plasminogen activator. The artery was tied distally, then proximally in situ to prevent dislodgment of the clot. At low power, residual thrombus is seen attached to the vessel wall (upper panels). Platelet aggregates (arrows) are seen at higher power and on electron microscopy (right lower panel).

cient fall in platelet count with 7E3 or OC125 (Table 2). These studies further demonstrate, therefore, a role for platelets in this model. Despite the inhibition of platelet aggregation by 7E3, there was evidence of continued platelet activation. Thus, urinary excretion of 2,3-dinor-TXB2 demonstrated a similar increase after reperfusion with t-PA in 7E3-treated (range 40–332%, median 150%) as in control experiments (range 31–443%, median 195%).

Discussion

Recent studies in experimental models of coronary thrombosis suggest that platelets limit the response to thrombolytic therapy.5,6,29 A possible mediator of platelet activation in this setting is TXA2. Thus, we have demonstrated a marked increase in TXA2 biosynthesis in patients with acute myocardial infarction treated with intravenous t-PA or streptokinase.8,9 These studies explore the role of TXA2 further and demonstrate that TXA2 limits the response to intravenous thrombolytic therapy and that this largely reflects TXA2-mediated platelet aggregation. Thus, 1) TXA2 biosynthesis was markedly increased after administration of t-PA in this platelet-dependent model of coronary thrombosis, 2) inhibition of TXA2 accelerated reperfusion and partly inhibited reocclusion, 3) the response depended on the degree of inhibition of TXA2, and 4) prevention of platelet aggregation exerted a similar effect without altering TXA2 biosynthesis.

Platelet activation has been shown to play a role in delaying reperfusion induced by t-PA5,6 and
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urinary excretion of 2,3-dinor-TXB2 (Tx-M) and 2,3-dinor-6-keto-PGF1α (PGI-M). Following the induction of coronary thrombosis, Tx-M and PGI-M increased (time = 0). A more marked increase occurred following the induction of reperfusion with tissue-type plasminogen activator (tPA).

Figure 4. Urinary excretion of 2,3-dinor-TXB2 (Tx-M) and 2,3-dinor-6-keto-PGF1α (PGI-M). Following the induction of coronary thrombosis, Tx-M and PGI-M increased (time = 0). A more marked increase occurred following the induction of reperfusion with tissue-type plasminogen activator (tPA).

streptokinase29 in a number of acute, open-chest models of coronary thrombosis. Interpretation of data obtained in such models is confounded by the marked platelet activation that occurs as a result of tissue trauma20 and that may exaggerate the platelet dependence of the response to t-PA. Thus, activated platelets release a number of proteins that may inhibit plasminogen activators, including a specific inhibitor, PAI-1.31 Experimental models have also suggested that platelet activation induces acute reocclusion.5,6,22 However, in these models, reocclusion occurred in the presence of a copper coil or a mechanically induced severe stenosis, both of which induce occlusion in the absence of pharmacological reperfusion. To avoid these artifacts, we used a chronic, closed-chest model in which coronary thrombosis was induced remote from the trauma of surgery. As in humans, the thrombus formed is rich in platelets and can be inhibited by antiplatelet drugs.15-17 Furthermore, reperfusion was complicated by a severe stenosis due to residual clot and by spontaneous reocclusion. A role for platelets was demonstrated biochemically and functionally. Thus, biosynthesis of TXA2, which is largely derived from platelets35 and is an index of platelet activation, increased markedly after reperfusion with t-PA. In addition, inhibition of platelet function demonstrated a role for platelets in this model. PGI2, a potent platelet inhibitor and vasodilator,34 prevented reocclusion at a dose that inhibited platelet aggregation ex vivo. That platelet activation, rather than vasoconstriction, played the predominant role in reocclusion was further supported by the results with two other antiplatelet strategies, TXA2 inhibition and blockade of the platelet GPIIb/IIIa. Unlike PGI2, these interventions have no hemodynamic effects.5,15,21 Both implicated platelets in delaying reperfusion and in inducing acute reocclusion.

As in humans, we demonstrated a marked increase in TXA2 biosynthesis during coronary thrombolysis in this model. To measure TXA2 formation, we determined the urinary excretion of its enzymatic metabolite, 2,3-dinor-TXB2, by gas chromatography and mass spectrometry. This is a highly specific assay and avoids the traumatic increase in TXA2 formation, which confounds measurements of its plasma hydrolysis product in TXE.35 In addition, TXA2 biosynthesis was determined in a chronic, stable state when eicosanoid formation, massively increased by surgery, had returned to normal.30 Urinary 2,3-dinor-TXB2 increased after reperfusion.

Figure 5. Urinary excretion of 2,3-dinor-TXB2 (Tx-M) and 2,3-dinor-6-keto-PGF1α (PGI-M) following coronary thrombolysis with tissue-type plasminogen activator (tPA). Administration of aspirin (ASA) 20 mg/kg i.v. immediately before tPA abolished the increase in Tx-M and PGI-M and decreased Tx-M.

Figure 6. Effect of PGI2 on coronary blood flow following reperfusion with tissue-type plasminogen activator (tPA) in the canine model. Following tPA administration, reperfusion was induced and was complicated by episodes of reocclusion. Heparin failed to prevent reocclusion despite marked prolongation of the partial thromboplastin time (PTT). In contrast, PGI2 prevented reocclusion and stabilized coronary flow. Complete reocclusion occurred following withdrawal of PGI2 (The step up in the tracing during PGI2 administration reflects resetting of the calibration to its pre-reperfusion value of 50 mv.)
TABLE 1. Comparison of Different Treatments on Plasma Fibrinogen, the Time to Reperfusion, and the Patency Rate Following Initial Reperfusion

<table>
<thead>
<tr>
<th></th>
<th>Time to occlusion (min)</th>
<th>Change in fibrinogen (%)</th>
<th>Time to reperfusion (min)</th>
<th>Patency &gt;1 hr postreperfusion (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>122±15 (9)</td>
<td>-13±3</td>
<td>60±5 (9)</td>
<td>0</td>
</tr>
<tr>
<td>PGI₃</td>
<td>130±14 (11)</td>
<td>-15±9</td>
<td>80±18 (6)</td>
<td>91*</td>
</tr>
<tr>
<td>ASA</td>
<td>124±14 (13)</td>
<td>-3±2</td>
<td>43±6 (6)</td>
<td>38</td>
</tr>
<tr>
<td>ASA+TRA</td>
<td>110±18 (11)</td>
<td>-18±9</td>
<td>26±3 (6)*</td>
<td>45*</td>
</tr>
</tbody>
</table>

PGI₃, prostacyclin; ASA, aspirin; TRA, TXA₂/prostaglandin endoperoxide-receptor antagonist.

Because L636,499 and AH 23848 induced a similar effect, they are considered together. Number in parentheses is the number of experiments. Drugs were given either before tissue-type plasminogen activator or after reperfusion to determine the effect of either method of administration on patency rates.

All values are mean±SEM.
*p<0.05 versus control.

and remained elevated over the 4 hours of observation. Furthermore, this increase was prevented by aspirin given immediately before reperfusion, demonstrating that this reflected de novo TXA₂ formation and not washout and subsequent metabolism of inactive TXB₂ formed during the induction of coronary thrombosis.

In addition to biochemical evidence of enhanced TXA₂ biosynthesis, these studies demonstrate a functional role for TXA₂ in this platelet-dependent model. Thus, inhibition of TXA₂ accelerated reperfusion and inhibited reperfusion, although incompletely. The dose of aspirin used in this study was selected to achieve a >95% inhibition of serum TXB₂ in the dog. This degree of inhibition of the capacity of platelets to generate TXA₂ is required to achieve substantial inhibition of TXA₂ formation in vivo. Despite marked suppression of TXA₂ biosynthesis, aspirin exerted only a modest effect on the response to t-PA. This may have reflected incomplete inhibition of TXA₂ biosynthesis since the response to aspirin was enhanced by the addition of either L636,499 or AH23848. L636,499 is a TXA₂/prostaglandin endoperoxide-receptor antagonist. Consistent with this, we have previously demonstrated that at the dose used in this study, L636,499 inhibited ex vivo platelet aggregation to the endoperoxide analogue, U46619, and to arachidonic acid but did not alter ADP-induced platelet aggregation. Furthermore, L636,499 had no effect

![Figure 7](http://example.com/figure7.png)

**Figure 7.** Effect of aspirin alone and combined with the TXA₂/prostaglandin endoperoxide-receptor antagonist, L636,499. In the upper tracing reperfusion was induced with tissue-type plasminogen activator (t-PA) and was complicated by episodic reocclusion and an increase in 2,3-dinor-TXB₂ (TxB₂). Aspirin (20 mg/kg) abolished cyclic flow coincident with a return of TxB₂ to baseline. In a separate experiment (lower panel), reperfusion was induced with t-PA and was complicated by reocclusion and a very marked increase in TxB₂. Aspirin (20 mg/kg) had little effect on either coronary flow or TxB₂, even 2 hours later. Addition of L636,499 stabilized coronary flow without a further reduction in TxB₂.
on platelet cyclic AMP, TXA₂, or PGI₂ biosynthesis. AH23848 is a structurally distinct TXA₂/prostaglandin endoperoxide–receptor antagonist, which at the doses used selectively inhibited U46619-induced platelet aggregation (data not shown). Thus, the additional effect of these antagonists of the TXA₂ receptor suggests residual TXA₂-mediated platelet activation. This was further supported by evidence of continued, although greatly reduced, TXA₂ biosynthesis following aspirin administration. Therefore, these studies demonstrate that a very marked degree of TXA₂ suppression is required to achieve a biological effect during coronary thrombolysis. Another mechanism that may have limited the response to aspirin was suppression of PGI₂ formation, as demonstrated by a reduction in the excretion of its enzymatic metabolite, 2,3-dinor-6-keto-PGF₁α. Indeed, PGI₂ may also play a role in modulating platelet activity in this setting. However, this is not the primary mechanism limiting the response to aspirin during coronary thrombolysis, since aspirin also enhanced the response to a TXA₂/prostaglandin endoperoxide–receptor antagonist, L636,499.

These studies therefore suggest a role for TXA₂ in limiting the response to thrombolytic therapy of coronary thrombosis. A similar effect was achieved by preventing platelet aggregation without inhibiting TXA₂ biosynthesis. Thus, 7E3, an antibody to both primate and canine platelet GPIIb/IIIa, the receptor for adhesive proteins required for platelet aggregation, abolished platelet aggregation, accelerated reperfusion, and prevented reocclusion. However, 7E3 did not inhibit TXA₂ formation, demonstrating that while it prevents platelet aggregation, it does not prevent primary activation of platelets. Consistent with this hypothesis, platelets exhibited a normal shape change to a variety of agonists ex vivo (Figure 8). This suggests that TXA₂-mediated platelet aggregation limits coronary thrombolysis.

Similarly, our findings suggest that the major mechanism of TXA₂-mediated reocclusion is plate...
let aggregation. In this study, reperfusion was complicated by a severe residual stenosis, which is also seen following coronary thrombolysis in humans,\(^{39}\) and was demonstrated angiographically to be the site of reocclusion. A number of investigators have demonstrated that platelet aggregation can induce occlusion at the site of a severe stenosis. This can be prevented in part by inhibition of TXA\(_2\)\(^{40-42}\) and totally by GPIIb/IIIa blockade.\(^{43}\) The primary stimulus to platelet activation in this setting is uncertain, but it may reflect an increased shear rate at the site of the stenosis. Consistent with this possibility, shear-induced platelet aggregation in vitro reflects part TXA\(_2\)-mediated expression of the GPIIb/IIIa binding site for adhesive proteins.\(^{44}\) In addition to platelet aggregation, reocclusion may reflect TXA\(_2\)-mediated vasoconstriction. TXA\(_2\) is a potent constrictor of canine vascular smooth muscle in vitro\(^{15}\) and has been shown to mediate platelet-dependent vasoconstriction at the site of vascular injury in vivo.\(^{32,45}\) This does not appear to be the primary mechanism of TXA\(_2\)-induced reocclusion since inhibition of platelet aggregation prevented reocclusion despite continued TXA\(_2\) formation. It is important to emphasize that even with very marked inhibition of TXA\(_2\), platelet-dependent reocclusion was not completely prevented, suggesting that there are other mechanisms of platelet activation following reperfusion as previously demonstrated.\(^{32}\) Furthermore, while not a primary mechanism of reocclusion in this model, vasoconstriction following reperfusion has been demonstrated in other models\(^{32}\) and in patients receiving intracoronary streptokinase.\(^{46}\)

In conclusion, these studies demonstrate that TXA\(_2\) may limit the response to coronary thrombolysis, both by delaying reperfusion and by inducing acute reocclusion, and that this appears to be mediated largely by platelet aggregation. Data from a large clinical trial demonstrating an enhanced effect of aspirin combined with streptokinase over streptokinase alone in patients with acute myocardial infarction are consistent with this hypothesis.\(^{47}\) Combined with evidence of increased platelet activation and TXA\(_2\) formation during coronary thrombolysis in humans, these data support clinical evaluation of the use of TXA\(_2\) inhibition early in the course of intravenous thrombolytic therapy in patients with acute myocardial infarction.

**Acknowledgment**

We gratefully acknowledge the technical assistance of Patricia Price.

**References**


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**Table 2. Comparison of the Effects of the F(ab')\(_2\) Fragments of the 7E3 Antibody to the Platelet Glycoprotein IIb/IIIa and OC125, the Control Antibody, in the Chronic Canine Model of Coronary Thrombolysis**

<table>
<thead>
<tr>
<th>Exp</th>
<th>Oclusion (min)</th>
<th>Reperfusion (min)</th>
<th>Reocclusion (min)</th>
<th>Fibrinogen (mg/dl) Pre-t-PA 2 hours</th>
<th>Platelet count (1,000/ul) Pre-t-PA 2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC125</td>
<td>202</td>
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<td>296±69</td>
<td>302±53</td>
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Platelet Activation and TXA<sub>2</sub> Response to t-PA


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thromboxane A₂ • tissue-type plasminogen activator
Increased thromboxane biosynthesis during coronary thrombolysis. Evidence that platelet activation and thromboxane A2 modulate the response to tissue-type plasminogen activator in vivo.

D J Fitzgerald, F Wright and G A FitzGerald

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