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Endogenous prostaglandin endoperoxides and prostacyclin modulate the thrombolytic activity of tissue plasminogen activator. Effects of simultaneous inhibition of thromboxane A2 synthase and blockade of thromboxane A2/prostaglandin H2 receptors in a canine model of coronary thrombosis.

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Research Article

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Endogenous Prostaglandin Endoperoxides and Prostacyclin Modulate the Thrombolytic Activity of Tissue Plasminogen Activator

Effects of Simultaneous Inhibition of Thromboxane A_2 Synthase and Blockade of Thromboxane A_2/Prostaglandin H_2 Receptors in a Canine Model of Coronary Thrombosis

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Abstract

We tested the hypothesis that simultaneous inhibition of TxA_2 synthase and blockade of TxA_2/PHG_2 receptors is more effective in enhancing thrombolysis and preventing reocclusion after discontinuation of tissue plasminogen activator (t-PA) than either intervention alone. Coronary thrombosis was induced in 35 dogs by placing a copper coil into the left anterior descending coronary artery. Coronary flow was measured with a Doppler flow probe. 30 min after thrombus formation, the animals received saline (controls, n = 10); SQ 29548 (0.4 mg/kg bolus + 0.4 mg/kg per h infusion), a TxA_2/PGH_2 receptor antagonist (n = 8); dazoxiben (5 mg/kg bolus + 5 mg/kg per h infusion), a TxA_2 synthase inhibitor (n = 9); or R 68070 (5 mg/kg bolus + 5 mg/kg per h infusion), a drug that blocks TxA_2/PGH_2 receptors and inhibits TxA_2 synthase (n = 8). Then, all dogs received heparin (200 U/kg) and a bolus of t-PA (80 μg/kg) followed by a continuous infusion (8 μg/kg per min) for up to 90 min or until reperfusion was achieved. The time to thrombolysis did not change significantly in SQ 29548-treated dogs as compared with controls (42 ± 5 vs. 56 ± 7 min, respectively, P = NS), but it was significantly shortened by R 68070 and dazoxiben (11 ± 2 and 25 ± 6 min, respectively, $P < 0.001$ vs. controls and SQ 29548-treated dogs). R 68070 administration resulted in a lysis time significantly shorter than that observed in the dazoxiben-treated group ($P < 0.01$). Reocclusion was observed in eight of eight control dogs, five of seven SQ 29548-treated dogs, seven of nine dazoxiben-treated dogs, and zero of eight R 68070-treated animals ($P < 0.001$). TxB_2 and 6-keto-$PGF_{1\alpha}$, measured in blood samples obtained from the coronary artery distal to the thrombus, were significantly increased at reperfusion and at reocclusion in control animals and in dogs receiving SQ 29548. R 68070 and dazoxiben prevented the increase in plasma TxB_2 levels, whereas 6-keto-$PGF_{1\alpha}$ levels were significantly increased with respect to control and SQ 29548-treated dogs. Thus, simultaneous inhibition of TxA_2 synthase and blockade of TxA_2/PGH_2 receptors is more effective than either intervention alone in this experimental model in enhancing thrombolysis and preventing reocclusion after t-PA administration. (*J. Clin. Invest.* 1990. 86:1095–1102.) Key words: thrombolysis • tissue plasminogen activator • prostaglandin endoperoxides • thromboxane A_2 synthase inhibitors • thromboxane A_2 receptor antagonists

Introduction

Early thrombolysis is now considered the treatment of choice for many patients with acute myocardial infarction. Attention has been focused on thrombolytic agents with a more pronounced thrombus selectivity, such as tissue-type plasminogen activator (t-PA).[1] t-PA offers the advantage of resulting in comparable reperfusion rates when given intravenously as compared with the intracoronary administration of streptokinase and to cause less extensive systemic fibrinogenolysis (1–7). However, despite these advantages of t-PA over streptokinase, some problems with t-PA as a thrombolytic agent remain. First, the incidence of reperfusion varies between 60 and 80% of patients (1–7). Second, the time necessary to achieve reperfusion from the beginning of t-PA administration is often at least 45 min (1–7). Finally, a substantial incidence of reocclusion, which exists after the adminstration of t-PA, is discontinued despite the use of heparin (1–7). Thus, substantial effort is being made to develop better thrombolytic agents and/or adjunctive therapies to be used in conjunction with currently available thrombolytic agents.

We (8) and others (9, 10) have demonstrated that intracoronary platelet activation plays a major role in causing early reocclusion after discontinuation of t-PA in experimental models of coronary thrombosis. Furthermore, intracoronary platelet activation and deposition on the thrombus may proceed during administration of t-PA, thus increasing the total amount of thrombus to be lysed and prolonging the time to reperfusion. We have shown that TxA_2 and serotonin cooperatively mediate these phenomena, as TxA_2 and serotonin antagonists given in combination, but not either intervention alone, markedly shorten the time to reperfusion and prevent or delay reocclusion after discontinuation of t-PA (11).

TxA_2, the major product of arachidonic acid metabolism

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1. *Abbreviations used in this paper:* ACT, activated coagulation time; LAD; left anterior descending coronary artery; t-PA, tissue plasminogen activator.

in platelets, is a potent platelet agonist and vasoconstrictor (12), and contributes in the pathophysiology of certain acute coronary artery disease syndromes (13). One way to overcome the deleterious effects of TxA_2 is by inhibiting TxA_2 synthase, the enzyme responsible for the conversion of PG endoperoxides (PGG_2/PGH_2) to TxA_2 (14). This approach has the advantage over cyclooxygenase inhibition of leaving the biosynthesis of prostacyclin (PGI_2) intact (15). PGI_2 is the major product of arachidonic acid metabolism in endothelial cells, and it is a potent anti-platelet substance (16). Indeed, the anti-platelet effects of TxA_2 synthase inhibitors may be potentiated by an increased production of anti-platelet prostaglandins formed by extra-platelet metabolism of PG endoperoxides that accumulate when TxA_2 synthase is inhibited (17, 18). However, PG endoperoxides may also activate a receptor on platelet membranes, shared with TxA_2, mediating platelet activation (19, 20). Thus, there may be conflicting influences as regards the anti-thrombotic activity of TxA_2 synthase inhibitors. Addition of a TxA_2 receptor antagonist to a TxA_2 synthase inhibitor should prevent the proaggregatory effects of PG endoperoxides, while leaving intact the increased production of anti-platelet prostaglandins, thus resulting in a potentiation of antithrombotic activity.

To determine whether these mechanisms might affect the thrombolytic activity of t-PA and the incidence of reocclusion after thrombolysis, we compared the effects of simultaneous inhibition of TxA_2 synthase and blockade of TxA_2/PGH_2 receptors on lysis time and incidence of reocclusion in a canine model of coronary thrombosis. This study provides direct evidence that this approach is more effective than either intervention alone in this model, and suggests a new class of agents with potential therapeutic importance during coronary thrombosis and thrombolysis.

Methods

Experimental preparation. 35 mongrel dogs were anesthetized with sodium pentobarbital, 30 mg/kg i.v. Respiration was maintained with a constant volume Harvard (Apparatus Co. Inc., S. Natick, MA) respirator. The right carotid artery and jugular vein were cannulated for monitoring systemic arterial pressure and administration of fluids and drugs, respectively. The heart was exposed through a left thoracotomy at the fifth intercostal space and suspended in a pericardial cradle. A segment of the left anterior descending coronary artery (LAD) was carefully isolated and a pulsed Doppler flow probe was placed around it. A small heparin-filled catheter was placed in a distal side branch of the LAD. Baseline hemodynamics, including those of heart rate, systemic blood pressure, and mean and phasic LAD blood flow velocity were recorded on a four-channel recorder (Hewlett-Packard Co., Palo Alto, CA; model 9270). Then, through a left carotid arterial cutdown, a No. 7F Amplatz L1 left coronary catheter (Cordis Laboratories Inc., Miami, FL) was positioned into the left coronary ostium under fluoroscopic control. A 0.025-inch, teflon-coated guidewire (Cook Co., Bloomington, IN) was positioned in the LAD and the catheter was removed. A copper coil of appropriate size made by wrapping a 24-gauge uncoated copper wire around needles of different sizes was positioned in the LAD immediately distal to the Doppler flow probe over the guidewire with the use of a flexible catheter tubing. Care was taken to make sure that no side branches originated from the LAD segment between the flow probe and the copper coil. The guidewire was then removed.

Protocol. Hemodynamic measurements were repeated after insertion of the coil. The coils are markedly thrombogenic and coronary thrombi promptly developed as documented by the flow probe. Presence of the intracoronary thrombus was documented for 30 min. The animals were then randomly assigned to one of the following groups: a group receiving saline (group I, $n = 10$) served as control; a group received a bolus of SQ 29548 (0.4 mg/kg), a potent and selective TxA_2/PGH_2 receptor antagonist (Squibb Pharmaceuticals, Princeton, NJ) (21, 22), followed by a continuous infusion (0.4 mg/kg per h) (group II, $n = 8$); a third group received a bolus of dazoxiben (5 mg/kg), a selective TxA_2 synthase inhibitor (Pfizer Pharmaceuticals, Groton, CT) (23), followed by a continuous infusion (5 mg/kg per h) (group III, $n = 9$); and a fourth group received a bolus of R 68070 (5 mg/kg), a new drug with simultaneous TxA_2/PGH_2 receptor blocking and TxA_2 synthase inhibiting properties (Janssen Research Foundation, Beerse, Belgium) (24, 25), followed by a continuous infusion (5 mg/kg per h) (group IV, $n = 8$). Immediately after starting drug or saline infusions, all dogs received a bolus of heparin (200 U/kg) and a bolus of 80 μg/kg of recombinant human tissue-type plasminogen activator (Knoll Pharmaceuticals Co., Whippany, NJ) followed by an infusion of 8 μg/kg per min for up to 90 min or until reperfusion was achieved. Heparin administration was eventually repeated every hour, whereas R 68070, SQ 29548, or dazoxiben infusion was maintained throughout the study. The time necessary to achieve adequate reperfusion (defined as LAD flow ≥ 70% of the baseline value), was identified and t-PA infusion was discontinued at that time.

Hemodynamics and LAD blood flow velocity were monitored continuously until a persistent reocclusion occurred (defined as a reocclusion lasting for at least 30 min) or until 90 min of total reperfusion time.

Prostaglandin measurements. To assess the effects of drug treatments on intracoronary PG production, TxB_2, the stable metabolite of TxA_2, and 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, were measured in blood samples obtained from the distal coronary catheter. Blood samples were taken before placement of the copper coil into the LAD 1 min after reperfusion was achieved, and at the moment of reocclusion or after 90 min of reperfusion. When a persistent reocclusion occurred, LAD blood flow approached zero and flow from the distal coronary catheter practically ceased. Therefore, blood samples at this time point were collected a few minutes before reocclusion, when LAD flow was ~ 30% of the baseline value. The distal coronary catheter was filled with heparin immediately after placement and after each sample was obtained. After discarding blood contained in the void space of the catheter, blood was allowed to flow directly into chilled Vacutainer tubes containing indomethacin (10 μg) and heparin (1,500 U). The flow rate from the distal coronary catheter was measured by dividing the volume of blood obtained by the collection time, which enabled calculations of intracoronary production of prostaglandins to be made (pg/ml plasma × ml/min = pg/min). Blood samples were centrifuged at 4°C at 2,000 *g* for 10 min, and the plasma was separated and frozen at −70°C until assayed.

TxB_2 and 6-keto-$PGF_{1\alpha}$ were measured by the method of Dray et al. (26) as modified by Campbell et al. (27). Briefly, after extraction and chromatographic purification, 0.1 ml of the sample was combined with 0.1 ml of ^3H-TxB_2 or ^3H-6-keto-$PGF_{1\alpha}$ and 0.1 ml of specific antiserum in a test tube. After incubation overnight at 4°C, the antibody-bound and antibody-free prostaglandins were separated with dextran-coated charcoal. The bound radioactivity was counted with a liquid scintillation spectrometer (Beckman Instruments, Palo Alto, CA).

Platelet aggregation. To evaluate the efficacy of the drug treatments in blocking TxA_2/PGH_2 receptors, platelet aggregation in response to U46619, a TxA_2 mimetic, was evaluated ex vivo. Blood samples were taken before insertion of the coil and after reperfusion was achieved, i.e., during drug infusion. 17 ml of blood were drawn in a syringe containing 3 ml of sodium citrate. Blood was centrifuged at 120 *g* for 20 min at room temperature to obtain platelet-rich plasma (PRP). PRP was removed and centrifuged at 1,000 *g* for 5 min to obtain platelet-poor plasma (PPP). Platelet aggregation was measured turbidimetrically on a Chrono-Log Corp. (Havertown, PA) aggregometer and recorded on a linear recorder. The aggregometer was calibrated with the use of PPP, and the test was performed on 250 μl of PRP in a

siliconized cuvette with continuous stirring. The platelet count in the PRP was adjusted to 300.000/μl by dilution with PPP as needed. Aggregation was induced in PRP in response to various concentrations of U46619. Since canine platelets do not aggregate in vitro in response to U46619 alone, the response of platelets to this agonist was recorded after priming them with subaggregatory concentrations of epinephrine (10 μM).

Fibrinolytic studies. Venous blood samples (4.5 ml) for measurements of fibrinogen, plasminogen, and alpha2-antiplasmin were collected in 0.5 ml (0.1 M) sodium citrate before insertion of the copper coil and immediately after stopping t-PA infusion. To inhibit activation of the fibrinolytic system in vitro, aprotinin was added at a final concentration of 200 kallikrein inhibitor U/ml of blood. Samples were placed on ice immediately and promptly centrifuged at 3,000 *g* for 10 min at 4°C. All plasma samples were transferred into polystyrene tubes and frozen at −20°C until assayed. 2-ml blood samples were also obtained before inserting the coil, at the moment of reperfusion, and at the moment of reocclusion or at 90 min of reperfusion to measure the activated coagulation time (ACT) using a Hemochron 400 (International Technidyne Co., Metuchen, NJ).

Statistical analyses. Results are expressed as mean±SE of the mean. Analysis of variance was used for multiple comparisons among groups. Differences for individual groups were tested with Student's *t* test for unpaired observations with Bonferroni's correction. Fisher's exact test was used to compare the occurrence of reocclusion in the various groups of animals. For comparisons of platelet aggregation data, fibrinolytic variables, prostaglandin measurements, hemodynamics, and LAD blood flows among groups, a two-way analysis of variance with a design for repeated measures was used.

Results

Induction of coronary thrombi and thrombolysis. Before positioning the copper coil into the coronary artery, LAD blood flow showed a stable and constant pattern (Fig. 1). The coil is markedly thrombogenic and usually induces coronary thrombosis within 2±1 min. Coronary blood flow approached undetectable values after formation of the intracoronary thrombus (Fig. 1).

Effective thrombolysis was achieved in eight of ten control dogs, eight of eight R68070-treated dogs, seven of nine SQ 29548-treated animals, and nine of nine dazoxiben-treated dogs. The animals that did not reperfuse at the end of the 90 min t-PA infusion were excluded from further statistical analyses. Effective thrombolysis, defined as a mean LAD blood flow ≥ 70% of the baseline value, was achieved in 56±7 min in control dogs (range 26–82 min, Fig. 2 *A*). SQ 29548 did not significantly reduce lysis time as compared with controls, 42±5 min (range 23–64, Fig. 2 *A*). Dazoxiben administration caused a significant reduction in lysis time as compared with control and SQ 29548-treated animals, 25±6 min (range 9–66, $P < 0.001$ vs. controls and SQ 29548 dogs, Fig. 2 *A*). Finally, R 68070 administration shortened lysis time to 11±2 min (range 5–19 min). Lysis time in this group was significantly shorter as compared with all other groups ($P < 0.001$ vs. control and SQ 29548-treated dogs, $P < 0.01$ vs. dazoxiben-treated animals, Fig. 2 *A*), thus indicating that R 68070 was more effective than SQ 29548 or dazoxiben in enhancing the thrombolytic properties of t-PA. As a consequence of shortened reperfusion time, the total dose of t-PA necessary to achieve thrombolysis in R 68070- and dazoxiben-treated dogs was also significantly reduced as compared with control and SQ 29548-treated animals (Fig. 2 *B*). Similar to reperfusion time, the total dose of t-PA necessary to achieve reperfusion in dazoxiben-treated dogs was significantly higher with respect to R 68070-treated animals (Fig. 2 *B*).

After successful thrombolysis (with the coil still in place), LAD blood flow showed a typical pattern in control dogs, characterized by gradual decreases of flow to almost zero values followed by spontaneous restorations of blood flow (cyclic flow variations). This pattern was observed for several minutes until a permanent reocclusion occurred (Fig. 1). Cyclic flow variations and a persistent reocclusion occurred in eight of eight control dogs that were successfully reperfused, and reocclusion time (defined as the time elapsed between the onset of reperfusion and occurrence of a persistent reocclusion) was 17±2 min (Fig. 3). SQ 29548 prevented the occurrence of cyclic flow variations and reocclusion in only two of the seven dogs that were successfully reperfused in this group

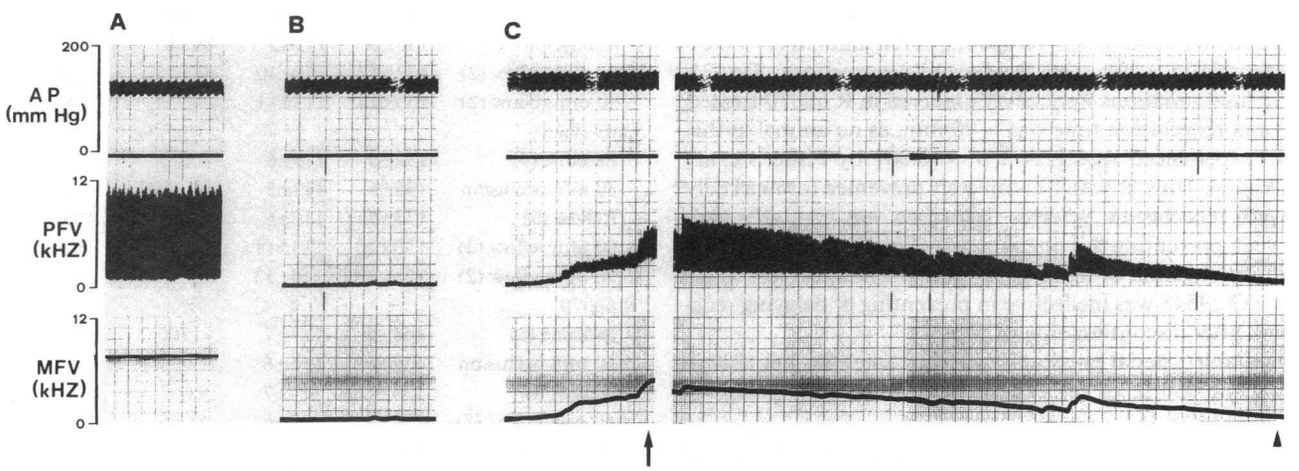

Figure 1. Representative tracing of hemodynamic data obtained from a control dog treated with heparin and t-PA (group I; *AP,* arterial pressure; *PFV,* peak flow velocity; *MFV,* mean flow velocity). (*A*) Before placement of the copper coil into the LAD, coronary blood flow shows a constant and stable pattern. (*B*) After positioning the coil, LAD blood flow decreased to unrecordable values. (*C*) During t-PA infusion, the intracoronary thrombus was progressively lysed and blood flow through the LAD was restored. The arrow indicates the time when the infusion of t-PA was discontinued. Despite the administration of 200 U/kg heparin, LAD blood flow showed a typical pattern of gradual decreases of flow followed by spontaneous restorations of flow until a persistent reocclusion occurred (*arrow head*).

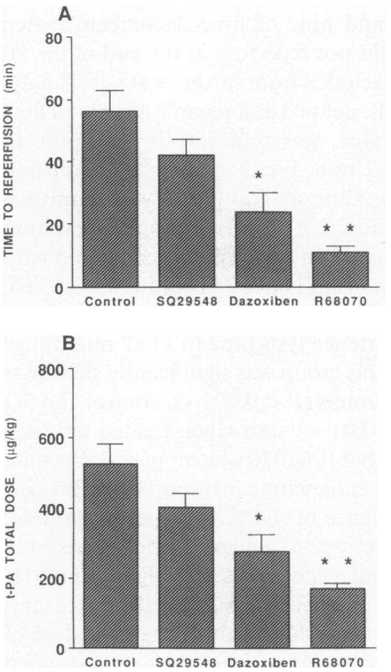

Figure 2. (*A*) Plot of time necessary to achieve thrombolysis by t-PA in dogs with coronary thrombosis receiving heparin (group I), heparin and SQ 29548 (group II), heparin and dazoxiben (Group III), and heparin and R 68070 (group IV). Only the administration of R 68070 or dazoxiben significantly decreased the time necessary to achieve effective thrombolysis. Furthermore, R 68070 administration resulted in a lysis time significantly shorter than that observed in dazoxiben-treated animals. $*P < 0.001$ vs. control and SQ 29548-treated dogs by *t* test with Bonferroni's correction; $**P < 0.01$ vs. dazoxiben-treated dogs by *t* test with Bonferroni's correction. (*B*) Plot of total dose of t-PA necessary to lyse the intracoronary thrombus in the same groups of animals. R 68070 or dazoxiben administration resulted in a significant reduction in the dose of t-PA necessary to achieve thrombolysis. In R 68070-treated animals, the total dose of t-PA was significantly reduced with respect to dazoxiben-treated animals. $*P < 0.001$ vs. control and SQ 29548-treated dogs; $**P < 0.01$ vs. dazoxiben-treated dogs.

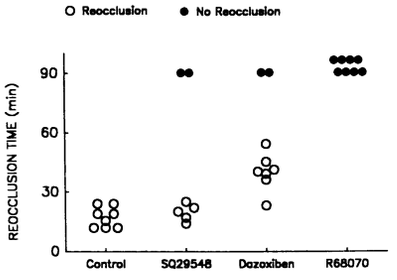

Figure 3. Plot of reocclusion time after discontinuation of t-PA infusion in dogs receiving heparin (group I), heparin and SQ 29548 (group II), heparin and dazoxiben (group III), and heparin and R 68070 (group IV). All animals treated with heparin alone (*Control*) reoccluded within 30 min after discontinuation of t-PA. SQ 29548 or dazoxiben prevented reocclusion in only two of seven dogs and two of nine dogs, respectively, whereas R 68070 administration prevented (or markedly delayed) reocclusion in all animals in this group throughout the experimental period of observation, i.e., 90 min. Dazoxiben caused a significant prolongation of reocclusion time with respect to control and SQ 29548-treated animals.

(P = NS vs. controls by Fisher's exact test) and reocclusion time was 20±4 min in the remaining five (P = NS vs. controls by *t* test with Bonferroni's correction; Fig. 3). Two of nine dazoxiben-treated dogs that were successfully reperfused did not show cyclic flow variations and did not reocclude throughout the experimental period (P = NS vs. controls by Fisher's exact test), but reocclusion time was significantly prolonged to 40±4 min in the remaining seven animals ($P < 0.01$ vs. controls by *t* test with Bonferroni's corrections; Fig. 3). Cyclic flow variations were never observed in R 68070-treated dogs and reocclusion time was > 90 min, as no animal in this group experienced reocclusion ($P < 0.001$ by Fisher's exact test; Fig. 3). Thus, R 68070 completely prevented (or markedly delayed) reocclusion, whereas dazoxiben was relatively ineffective in preventing this phenomenon, but resulted in a significant prolongation of reocclusion time with respect to control dogs. SQ 29548 was ineffective in preventing or delaying reocclusion after discontinuation of t-PA.

Systemic arterial pressures and heart rates did not change during the course of the experiments in the four groups of animals (Table I).

A certain degree of bleeding was observed from the surgical wounds, which was never severe enough to significantly decrease arterial blood pressure.

Prostaglandin measurements. Fig. 4 shows the intracoronary production of TxB_2 and 6-keto-$PGF_{1\alpha}$ at baseline (i.e., before placement of the intracoronary copper coil), at reperfusion, and at the moment of reocclusion or 90 min after reperfusion. TxB_2 production in the coronary artery increased significantly in control animals from a baseline value of 891±78 to 3,380±228 pg/min at reperfusion and 3,549±208 pg/min at reocclusion ($P < 0.001$). Treatment with R 68070 prevented the increase in intracoronary TxB_2 production and actually decreased TxB_2 production from a baseline value of 988±111

Table I. Hemodynamic Variables before and after Thrombolysis with t-PA

	HR	AOM	PHF	MNF
	b/min	*mmHg*	*% of control*	*% of control*
Control				
Baseline (10)	133±6	111±4	100	100
30 min occlusion	134±7	117±4	0.7±0.4*	0.7±0.3*
Reflow (7)	131±5	112±4	91±6	102±6
60 min reflow (0)	—	—	—	—
SQ 29548				
Baseline (9)	137±6	112±7	100	100
30 min occlusion	139±7	118±8	0.6±0.5*	0.5±0.4*
Reflow (7)	136±8	115±5	89±8	94±7
60 min reflow (2)	132±12	111±10	86±12	88±11
90 min reflow (2)	133±13	113±11	85±11	86±10
Dazoxiben				
Baseline (9)	132±5	115±6	100	100
30 min occlusion	135±6	112±5	0.7±0.5*	0.6±0.4*
Reflow (9)	133±7	115±6	93±8	103±7
60 min reflow (2)	130±10	113±11	89±12	91±11
90 min reflow (2)	128±12	110±13	87±13	90±13
R 68070				
Baseline (8)	140±5	117±7	100	100
30 min occlusion	139±8	115±6	0.8±0.4*	0.5±0.3*
Reflow (8)	134±7	114±7	91±7	98±6
60 min reflow (8)	138±7	118±6	86±5	90±4
90 min reflow (8)	138±6	114±6	89±3	90±3

HR, heart rate; AOM, mean aortic pressure; PHF, left anterior descending coronary artery peak flow velocity; MNF, left anterior descending coronary artery mean flow velocity. Numbers in parentheses refer to the number of animals included in that time period.
* $P < 0.05$ vs. baseline values.

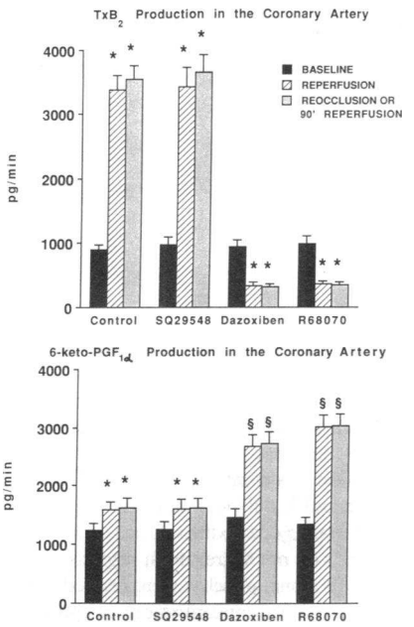

Figure 4. (*Top*) TxA_2 production in the distal coronary artery at baseline, at the moment of reperfusion, and at reocclusion or 90 min after reperfusion in control dogs and in dogs receiving SQ 29548, dazoxiben, or R 68070. A marked increase in TxB_2 production was observed in control and SQ 29548-treated animals at reperfusion and at reocclusion. Both dazoxiben and R 68070 resulted in a significant decrease in intracoronary TxB_2 production. $*P < 0.001$ vs. the corresponding baseline value. (*Bottom*) 6-keto-$PGF_{1\alpha}$ production in the distal coronary artery at baseline, at reperfusion, and at reocclusion or 90 min after reperfusion in control dogs or in dogs receiving SQ 29548, dazoxiben, or R 68070. Similar to intracoronary TxB_2 production, an increase in intracoronary 6-keto-$PGF_{1\alpha}$ production was observed in control and SQ 29548-treated dogs at reperfusion and at reocclusion. Both dazoxiben and R 68070 markedly increased the intracoronary production of this prostaglandin with respect to control and SQ 29548-treated dogs. $*P < 0.01$ vs. the corresponding baseline values; § $p < 0.001$ vs. control and SQ 29548-treated dogs.

to 356±56 pg/min at reperfusion and 348±50 pg/min 90 min after reperfusion was achieved. Similar findings were observed in dazoxiben-treated dogs (Fig. 4 *A*). In contrast, SQ 29548 did not affect TxA_2 synthesis, as intracoronary TxB_2 production increased at reperfusion and at reocclusion to a similar extent as that found in control animals (Fig. 4 *A*). Intracoronary production of 6-keto-$PGF_{1\alpha}$ also increased significantly in control dogs at reperfusion and at reocclusion with respect to baseline values (Fig. 4 *B*). In addition to decreasing intracoronary TxB_2 production, both R 68070 and dazoxiben significantly increased 6-keto-$PGF_{1\alpha}$ production in the coronary artery in comparison to control animals at reperfusion and at reocclusion or 90 min after reperfusion (Fig. 4 *B*).

Table II summarizes the 6-keto-$PGF_{1\alpha}/TxB_2$ ratio in the distal coronary artery at baseline, reperfusion, and at the moment of reocclusion or after 90 min of reperfusion. This index is independent of changes in coronary flow and significantly decreased at reperfusion and at the moment of reocclusion both in control and SQ 29548-treated animals. In contrast, this ratio significantly increased at the same time periods in R 68070 and dazoxiben-treated dogs.

Table II. Effects of Drug Treatments on 6-keto-$PGF_{1\alpha}/TxB_2$ Ratio in Blood Collected from the Distal Coronary Artery

	Baseline	Reperfusion	Reocclusion or 90′ reperfusion
Control	1.5±0.2	0.5±0.1*	0.5±0.1*
SQ 29548	1.3±0.1	0.5±0.1*	0.5±0.1*
Dazoxiben	1.7±0.3	9.8±1.6*‡	10.2±1.9*‡
R 68070	1.5±0.3	10.6±2.8*‡	10.0±2.0*‡

* $P < 0.01$ vs. baseline; ‡ $P < 0.001$ vs. corresponding value in Control or SQ 29548-treated group.

Fibrinolytic studies. ACTs are summarized in Fig. 5. ACTs were similar in the four experimental groups at baseline and were significantly prolonged at reperfusion and at reocclusion or 90 min after reperfusion with respect to baseline, but no differences were observed among the four groups. Fibrinogen, plasminogen, and $alpha_2$-antiplasmin levels significantly decreased after administration of t-PA to a similar extent in the four groups of animals with respect to baseline. No differences were observed among the four groups at baseline and after t-PA administration (Table II). Thus, the observed differences in lysis and reocclusion times among the four groups cannot be explained by differences in fibrinogen, plasminogen, and $alpha_2$-antiplasmin levels or ACTs.

Platelet aggregation. Ex vivo platelet aggregation in response to various concentrations of U46619 was blocked during infusion of R 68070 and SQ 29548. On the other hand, dazoxiben did not alter platelet response to U46619, thus indicating that R 68070 and SQ 29548, at the doses used, were effective in blocking TxA_2/PGH_2 receptors on platelets and that dazoxiben is not a TxA_2/PGH_2 receptor antagonist (Fig. 6).

Discussion

The present study addresses the efficacy of different interventions that interfere with the synthesis and/or the receptors of TxA_2 in enhancing the thrombolytic activity of tissue plasminogen activator in a canine model of coronary thrombosis. Specifically, we hypothesized that simultaneous blockade of TxA_2/PGH_2 receptors and inhibition of TxA_2 synthase is more effective in enhancing thrombolysis and preventing reocclusion after discontinuation of t-PA than either intervention alone. The experimental preparation used in the present study is platelet-dependent in that it is associated with intracoronary platelet activation, increased TxA_2 biosynthesis, and inhibition by antiplatelet agents (8, 11).

To test this hypothesis, we have studied the effects of SQ 29548, a selective TxA_2 receptor antagonist; dazoxiben, a selective TxA_2 synthase inhibitor; and R 68070, a new drug with simultaneous TxA_2 synthase inhibiting and receptor antagonist properties. Consistent with its pharmacological properties (21, 22), SQ 29548 did not inhibit intracoronary TxA_2 produc-

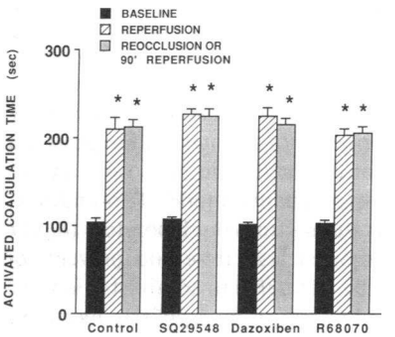

Figure 5. ACTs in the various groups. ACTs were significantly prolonged in all groups at reperfusion and at reocclusion or after 90 min of reperfusion with respect to baseline values, but no differences were found among groups.

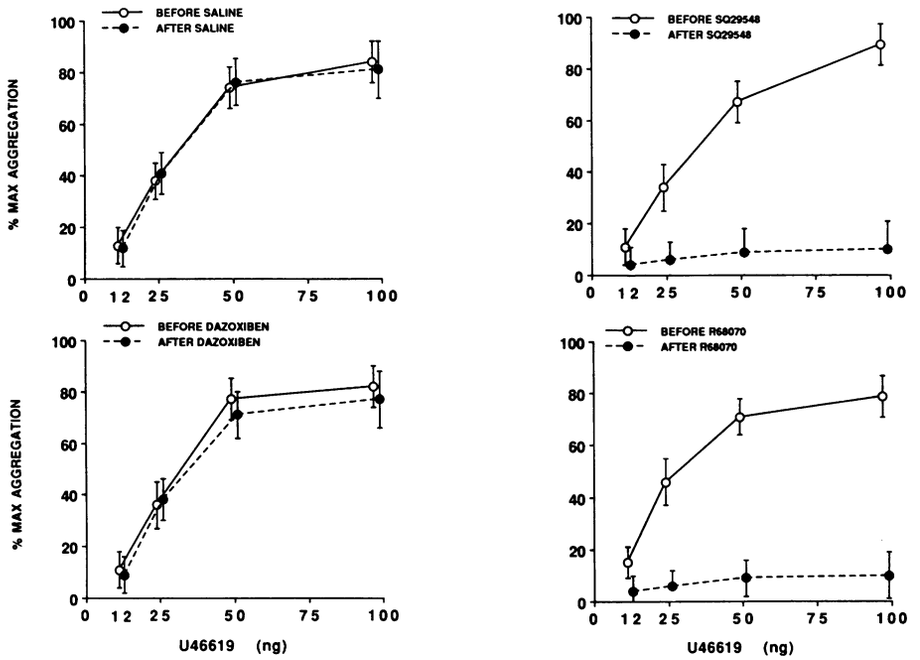

Figure 6. Ex vivo platelet aggregation in response to various concentrations of U46619 (a TxA_2 mimetic) before and during infusion of saline (control group), SQ 29548, dazoxiben, or R 68070. Platelet aggregation was completely inhibited by SQ 29548 and R 68070, whereas it was largely unaffected by dazoxiben. Since canine platelets do not aggregate in response to U46619 alone, platelets were primed with subaggregatory concentrations of epinephrine (10 μM).

tion at reperfusion and during reocclusion, but it did prevent ex vivo platelet aggregation in response to the TxA_2 analogue, U46619. Dazoxiben is a potent TxA_2 synthase inhibitor (23), and it suppressed intracoronary TxA_2 production at reperfusion and during reocclusion, whereas it had no effect on U46619-induced platelet aggregation. R 68070 is a new drug that possesses simultaneous TxA_2 synthase inhibiting and TxA_2/PGH_2 receptor blocking properties (24, 25). Indeed, at the dose employed in the present study, R 68070 resulted in a simultaneous blockade of TxA_2/PGH_2 receptors and inhibition of TxA_2 synthase, as U46619-induced platelet aggregation was inhibited and intracoronary TxA_2 production suppressed to a similar extent as noted in SQ 29548- and dazoxiben-treated animals.

The three interventions used in the present study affected lysis time and the rate of reocclusion differently. SQ 29548, despite its potent effects on U46619-induced platelet aggregation, did not significantly shorten lysis time or prevent reocclusion after discontinuation of t-PA. In contrast, dazoxiben caused a significant reduction in lysis time as compared with controls and SQ 29548-treated dogs. Furthermore, dazoxiben significantly prolonged reocclusion time with respect to controls and SQ 29548-treated animals. Simultaneous blockade of TxA_2/PGH_2 receptors and inhibition of TxA_2 synthase by R 68070, greatly enhanced the thrombolytic properties of t-PA in this experimental model, as lysis time was significantly shorter than that measured in all other groups. Moreover, reocclusion was prevented (or markedly delayed) in eight of eight dogs in this group and reocclusion time was greater than 90 min.

The data from the present study demonstrate that blockade of TxA_2/PGH_2 receptors alone is not an intervention powerful enough to yield a significant reduction in lysis time and to prevent reocclusion in this experimental model. This finding is in agreement with previous studies from our laboratory (8, 11). Failure of SQ 29548 to shorten lysis time and prevent (or delay) reocclusion can not be attributed to administration of a dose too low to achieve blockade of TxA_2/PGH_2 receptors, since ex vivo platelet aggregation in response to U46619 was inhibited. A possible explanation for the lack of protective effect from the TxA_2/PGH_2 receptor antagonists alone is that platelets are activated in vivo in response to a variety of substances, including TxA_2, thrombin, collagen, ADP, serotonin, and epinephrine. Each of these agonists has its own receptors on platelet membranes and each causes receptor-linked signal transduction which results in platelet activation. One can easily block one of the major pathways with a pharmacological agent, but even if one pathway is blocked, other pathways may prevail, such that platelet activation still occurs. Indeed, in two recent studies from our laboratory, we have demonstrated that in order to reduce lysis time significantly and prevent (or markedly delay) reocclusion, one needs to simultaneously block two of the major pathways of platelet activation, namely TxA_2 and serotonin (8, 11).

Data from the present study also suggest that the response to dazoxiben (a TxA_2 synthase inhibitor) was limited by either incomplete inhibition of TxA_2 formation or by the accumulation of prostaglandin endoperoxides. Prostaglandin endoperoxides, precursors of TxA_2 in the cyclooxygenase pathway of arachidonic acid metabolism in platelets, activate a receptor shared with TxA_2 that induces platelet activation and vascular smooth muscle contraction (19). In vitro studies demonstrate that prostaglandin endoperoxides may substitute for the proaggregatory effect of TxA_2 and overcome the effect of TxA_2 synthase inhibition on platelets (19, 20, 28). In view of the marked inhibition of intracoronary TxA_2 production by dazoxiben in this study, these data are consistent with the hypothesis that prostaglandin endoperoxides may limit the response to t-PA in this experimental model.

The present study also demonstrates that simultaneous blockade of TxA_2/PGH_2 receptors and inhibition of TxA_2 synthase (obtained by R 68070) is more effective than either intervention alone in enhancing thrombolysis and preventing reocclusion after discontinuation of t-PA. In part, this may reflect a further reduction of TxA_2 formation. Alternatively, it

may be due to increased biosynthesis of platelet inhibitory prostaglandins, including PGI_2 (17, 18) and PGD_2 (29, 30) by endothelial cells and by the isomerase activity of plasma albumin, respectively. Indeed, this may be a major mechanism of action of TxA_2 synthase inhibitors both in vitro (30) and in vivo (31). As evidence of a shunt of prostaglandin endoperoxides toward platelet inhibitory prostaglandins, there was a marked increase in intracoronary PGI_2 production, coincident with the inhibition of TxA_2 biosynthesis in animals treated with R 68070 and dazoxiben. This effect was most marked at the time of reperfusion and during rethrombosis, when platelet activation is markedly increased. Similar findings have recently been reported by Fitzgerald et al. in a chronic model of coronary thrombosis (32). Another finding supporting this hypothesis has been reported in a recent study from our laboratory, in which combined TxA_2 synthase inhibition and receptor blockade (by R 68070) was more effective in preventing epinephrine-induced cyclic flow variations than blockade of TxA_2/PGH_2 receptors alone (33). This protective effect of R 68070 was lost when PGI_2 production was inhibited by aspirin given before R 68070 (33).

The ultimate goal of coronary thrombolysis as a treatment of acute myocardial infarction is to reduce the extent of ischemic myocardium that will ultimately undergo necrosis,thus preserving left ventricular function and reducing mortality. However, the potential benefits of coronary thrombolysis may be significantly reduced or even offset by (*a*) an excessively long time delay between onset of ischemia and achievement of reperfusion, and (*b*) reocclusion of the infarct-related artery.

It has been shown that arterial thrombi continue to grow after their formation by incorporation of new fibrin (34) and adhesion of new platelets (35). Activated platelets bind fibrin (36) through specific receptors associated with the glycoprotein IIb/IIIa complex and non-specific binding (37). Thrombus growth is particulary rapid during the first few hours (34, 35) and may proceed during administration of thrombolytic agents, thus increasing the total amount of thrombus to be lysed. This phenomenon may delay recanalization of the infarct-related artery and reduce the potential for myocardial salvage. The balance between these two opposing processes may influence the clinical outcome of thrombolytic treatment. Interfering with formation of new fibrin and/or activation and deposition of new platelets on the thrombus during administration of t-PA may favorably shift this balance toward a potentiation of the lytic properties of t-PA.

For example, heparin has been shown to prevent new fibrin formation and its incorporation into the thrombus, enhancing the thrombolytic effect of t-PA (38). In a recent study, Gold et al. demonstrated that inhibition of platelet adhesion to the thrombus, by administration of a selective monoclonal antibody directed against the platelet GP IIb/IIIa complex in combination with t-PA, accelerates thrombolysis and prevents early reocclusion (9). In recent studies from our laboratory, in the same animal model used in the present study, we demonstrate that thromboxane A_2 and serotonin are important mediators of platelet activation and reocclusion after discontinuation of t-PA (8). We have also demonstrated that thromboxane A2 and serotonin antagonists given in combination before t-PA infusion markedly shorten the time to reperfusion and prevent or delay reocclusion after discontinuation of t-PA (11).

In conclusion, the present study demonstrates that a significant enhancement of thrombolysis by t-PA can be achieved by simultaneous blockade of TxA_2/PGH_2 receptors and inhibition of TxA_2 synthase with respect to either intervention alone, and that this treatment is also able to prevent early reocclusion in this experimental model. Prostaglandin endoperoxides appear to modulate the response to t-PA in this experimental model of coronary thrombosis, and they may limit the efficacy of t-PA by substituting for the proaggregatory effects of TxA_2. However, in the presence of an inhibitor of TxA_2 synthase and a TxA_2/PGH_2 receptor antagonist, they can be metabolized to platelet inhibitory prostaglandins. The functional importance of this shunting toward the synthesis of anti-platelet prostaglandins can be demonstrated when the proaggregatory effects of prostaglandin endoperoxides are eliminated by simultaneous blockade of the shared TxA_2/PGH_2 receptors. The resulting synergistic interaction between TxA_2/PGH_2 receptor blockade and inhibition of TxA_2 synthase obtained by R 68070 renders this compound an effective anti-platelet drug of potential therapeutic importance. Whether this beneficial effect may apply to patients with acute myocardial infarction receiving thrombolytic therapy is not yet clear, but this possibility should be evaluated.

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