Simultaneous Administration of Thromboxane $\text{A}_2$– and Serotonin $\text{S}_2$–Receptor Antagonists Markedly Enhances Thrombolysis and Prevents or Delays Reocclusion After Tissue-Type Plasminogen Activator in a Canine Model of Coronary Thrombosis

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Dynamic changes of the thrombus after its formation due to platelet activation may affect the speed of thrombolysis. In the present study, we wanted to evaluate the role played by thromboxane $\text{A}_2$ (TXA$_2$) and serotonin (5HT) in mediating platelet activation during lysis of intracoronary thrombi with human recombinant tissue-type plasminogen activator (t-PA). Coronary thrombi were induced in 26 anesthetized, open-chest dogs by inserting a copper coil into the left anterior descending coronary artery (LAD). LAD blood flow was monitored throughout the experiment by means of a Doppler flow probe placed proximally to the coil. Presence of the thrombus was documented for 30 minutes. The dogs were then assigned to one of four groups as follows: group 1 dogs ($n=8$), serving as controls, received a bolus of heparin (200 units/kg) and a bolus of t-PA (80 $\mu$g/kg) followed by a continuous infusion (8 $\mu$g/kg/min) for up to 90 minutes or until reperfusion was achieved; group 2 dogs ($n=10$) received, immediately before heparin and t-PA, an intravenous bolus of SQ29548 (SQ) (0.4 mg/kg, a selective TXA$_2$-receptor antagonist) and LY53857 (LY) (0.2 mg/kg, a selective serotonin S$_2$–receptor antagonist); group 3 dogs ($n=7$) received, before heparin and t-PA, an intravenous bolus of SQ alone (0.4 mg/kg); and group 4 dogs ($n=7$) received, before heparin and t-PA, an intravenous bolus of LY alone (0.2 mg/kg). After thrombolysis, all dogs were monitored for 90 minutes or until a persistent reocclusion occurred. Treatment with the combination of SQ and LY markedly shortened the time required to lyse the thrombus, 46±7 minutes compared with 15±3 minutes in groups 1 and 2, respectively, $p<0.01$; whereas either antagonist alone was not effective in accelerating the lysis, 38±8 and 43±4 minutes in groups 3 and 4, respectively, $p=\text{NS}$ compared with group 1. In group 1 dogs, after discontinuation of t-PA, repeated cycles of gradual occlusions followed by spontaneous restorations of flow (cyclic flow variations, CFVs) were observed before a persistent reocclusion occurred. The administration of both SQ and LY immediately before heparin and t-PA completely prevented CFVs and reocclusion in all the group 2 dogs that were successfully reperfused. Neither SQ nor LY alone was effective in preventing CFVs and reocclusion. We conclude that SQ and LY in combination markedly enhance the thrombolytic effect of t-PA, probably by preventing further platelet activation and their incorporation into the thrombus during lysis. Furthermore, the combination of the two drugs is very effective in preventing reocclusion after discontinuation of t-PA. (Circulation 1989;79:911–919)
Early coronary thrombolysis is being evaluated extensively as a means to prevent or attenuate the pathophysiologic events occurring with acute myocardial infarction. Several studies have shown that administration of tissue-type plasminogen activator (t-PA) is associated with a high rate of successful thrombolysis in patients with acute myocardial infarction.1-5 The major problem emerging from these studies was a relatively high rate of reocclusion after the administration of t-PA was discontinued,1-5 and this may limit the efficacy of thrombolysis. We have recently demonstrated that, in a canine model of coronary thrombosis, reocclusion after discontinuation of t-PA is platelet dependent and is mediated by thromboxane A2 (TXA2) and serotonin (5HT).6

Another factor that may affect the efficacy of thrombolysis is the rapidity with which adequate reperfusion of the ischemic myocardium occurs. Experimental studies conducted in dogs demonstrated that beyond 3-4 hours, reperfusion does not result in appreciable myocardial salvage.7 Similarly, recovery of ventricular function is also critically dependent on the duration of the ischemic period.8,9

It has been shown that arterial thrombi are in a dynamic state for several hours after their formation with new fibrin and platelets being added to the thrombus, especially during the first few hours.10,11 Therefore, it is possible that dynamic changes in the thrombus affect the efficacy of thrombolytic agents by increasing the total amount of thrombus to be lysed. For example, Cercek et al12 demonstrated that pretreatment with heparin enhances the thrombolytic effects of t-PA by blocking the deposition of new fibrin into the thrombus, and there is also evidence from several preliminary reports that the administration of prostaglandin E1, which inhibits platelet activation, markedly accelerates the thrombolytic effect of urokinase, streptokinase, and t-PA both in the experimental setting and in patients with myocardial infarction.13,15 Furthermore, a recent study by Gold et al16 has shown that administration of a monoclonal antiplatelet GPIb/IIa antibody before t-PA injections significantly shortens the time to reperfusion and prevents reocclusion after discontinuation of thrombolytic therapy in a canine preparation of coronary thrombosis.

Platelets are activated in response to a variety of substances. In particular, several studies from our laboratory have demonstrated that TXA2 and 5HT are important mediators of intracoronary platelet activation in an experimental preparation of concentrically stenosed canine coronary arteries and endothelial injury.17-20 Accordingly, the present study was performed to investigate the role played by TXA2 and 5HT in mediating platelet activation and dynamic changes of the thrombus during administration of t-PA, thus affecting the speed of thrombolysis. To achieve this goal, SQ29548, a selective TXA2-PGHI receptor antagonist, and LY53857, a selective 5HT1 receptor antagonist, were administered at the moment of initiating thrombolytic therapy rather than at the moment of reperfusion.

Methods

Experimental Preparation

This study was performed in 32 open-chest dogs of 20-32 kg body wt. Dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.), intubated, and ventilated with room air with a Harvard respirator. Polyethylene catheters were inserted throughout the right carotid artery and a jugular vein for monitoring systemic arterial pressure and administration of fluids and drugs, respectively. A left thoracotomy was performed at the fifth intercostal space, and the heart was suspended in a pericardial cradle. A segment of the left anterior descending coronary artery (LAD) was carefully isolated from the surrounding tissue, and a pulsed Doppler flow probe was placed around it.21 Baseline hemodynamics, including those of heart rate, systemic blood pressure, and mean and phasic LAD blood flow velocities, were recorded on a four-channel recorder (model 9270, Hewlett-Packard). Then, through a left carotid arterial cutdown, a 7F Amplatz L1 left coronary catheter (Cordis, Miami, Florida) 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 ensure that no side branches originated from the LAD segment between the flow probe and the copper coil. The guidewire was then removed. The presence of the intracoronary thrombus was associated with no detectable LAD flow and systolic bulging of the myocardium supplied by the LAD.

Protocol

Hemodynamic measurements were repeated and, after a 30-minute waiting period to document persistence of the intracoronary thrombus, the animals were initially assigned to one of two groups. Group 1 dogs (n=8), serving as controls, received a bolus of heparin (200 units/kg) and a bolus of 80 μg/kg human recombinant t-PA (Knoll Pharmaceuticals, Whippany, New Jersey) followed by an infusion of 8
μg/kg/min for up to 90 minutes or until reperfusion was achieved. Group 2 dogs (n=10) received, immediately before heparin and t-PA, an intravenous bolus of SQ29548 (0.4 mg/kg) (Squibb Pharmaceuticals, Princeton, New Jersey), a potent and selective thromboxane A₂-prostaglandin H₂-receptor antagonist²²,²³ and an intravenous bolus of LY53857 (0.2 mg/kg) (Eli Lilly Pharmaceuticals, Indianapolis, Indiana), a selective serotonin S₂-receptor antagonist.²⁴ The time necessary to achieve adequate reperfusion (defined as LAD flow of 70% or more of the baseline value, at which time t-PA infusion was discontinued) was carefully measured. Hemodynamics and LAD blood flow were monitored continuously until a reocclusion occurred (in which case the observation period was extended for 30 additional minutes to ensure that the thrombus was persistent) or up to 90 minutes. After these initial experiments were performed, we wanted to determine the effect of either antagonist alone on lysis and reocclusion time. Accordingly, 14 animals were assigned to one of two additional groups. Group 3 dogs (n=7) received, immediately before heparin and t-PA, an intravenous bolus of SQ29548 alone (0.4 mg/kg). Group 4 dogs (n=7) received, before heparin and t-PA, an intravenous bolus of LY53857 alone (0.2 mg/kg). Dogs in groups 3 and 4 were subjected to the same protocol described for group 1 and 2 dogs.

**Fibrinolytic Studies**

Venous blood samples (4.5 ml) for measurements of fibrinogen were collected in 0.5 (0.1 M) sodium citrate before inserting the copper coil and immediately after stopping the t-PA infusion. Samples were placed on ice immediately and promptly centrifuged at 3,000g for 10 minutes at 4°C. To inhibit activation of the fibrinolytic system in vitro, the plasma was then transferred into polystyrene tubes supplemented with aprotinin at a final concentration of 200 kallikrein inhibitor units/ml plasma. All plasma samples were frozen at 20°C until assayed. Two-millimeter blood samples were also obtained before inserting the coil, at the moment of reperfusion, and at the moment of reocclusion or at 90 minutes of reperfusion to measure the activated coagulation time (ACT) with a Hemochron 400 (International Technidyne, Monroeville, New Jersey). Fibrinogen levels were determined as total coagulable protein by the method of Ratnoff and Menzie.²⁵

**Statistical Analyses**

Results are expressed as mean ± SD unless otherwise specified. 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 the Bonferroni’s correction. Fisher’s exact test was used to compare the occurrence of reocclusion in the various groups. For comparisons of hemodynamics and LAD blood flows among groups, a two-way analysis of variance for a design with repeated measures was used.

**Results**

**Induction of Coronary Thrombi and Thrombolysis**

Before placement of the copper coil into the coronary artery, LAD blood flow showed a stable and constant pattern (Figure 1A). The coils are markedly thrombogenic and consistently induced
coronary thrombosis within 2±1 minutes (range, 0–7 minutes). Coronary blood flow approached zero after positioning of the coil and thrombus formation (Figure 1B).

Effective thrombolysis was achieved in five of eight group 1 dogs, nine of 10 group 2 dogs, and six of seven group 3 dogs. The animals that did not reperfuse at the end of the 90-minute t-PA infusion were excluded from further statistical analysis. The time required to achieve effective thrombolysis (defined as a mean LAD flow of 70% or more of the baseline value) averaged 46±14 minutes in group 1 dogs (controls) (range, 26–64 minutes) (Figure 2). The administration of both SQ29548 and LY53857 to group 2 dogs, immediately before heparin and t-PA, induced lysis of the intracoronary thrombus in 15±7 minutes (range, 2–23 minutes, \( p<0.005 \) compared with group 1 by \( t \) test with Bonferroni’s correction), whereas the administration of either SQ29548 or LY53857 immediately before heparin and t-PA did not shorten the time required to lyse the thrombus, 43±14 and 40±7 minutes in group 3 and 4 dogs, respectively; \( p=\text{NS} \) compared with group 1 by \( t \) test (Figure 2A). This resulted in a significant reduction in the total dose of t-PA administered in group 2 dogs as compared with all other groups, \( p<0.005 \) by \( t \) test (Figure 2B).

After successful thrombolysis (Figure 1C) and with the coil still in place, LAD blood flow showed a typical pattern characterized by gradual decreases of flow to almost zero values followed by spontaneous restorations of blood flow (cyclic flow variations, CFVs). This pattern was observed for several minutes until a permanent reocclusion occurred (Figure 1C). Reocclusion time, defined as the time elapsed between the onset of reperfusion and the occurrence of a persistent reocclusion, was 17±5 minutes in the five group 1 dogs that reperfused (Table 1). Within 25 minutes, all group 1 dogs reoccluded. The administration of both SQ29548 and LY53857 immediately before heparin and t-PA prevented CFVs and the occurrence of reocclusion in all the nine group 2 dogs that were successfully reperfused (\( p<0.005 \) compared with groups 1, 3, and 4 by Fisher’s exact test) (Table 1). In these animals, LAD blood flow remained stable throughout the reperfusion period (i.e., 90 minutes) (Figure 3). Reocclusion was prevented in only one dog in group 3 and one in group 4 (\( p=\text{NS} \)) (Table 1), thus showing that either antagonist alone was not effective in preventing occlusion.

Activated coagulation times (ACTs) are summarized in Table 1. ACTs were similar in all groups at baseline and were significantly prolonged before reperfusion in all groups with respect to baseline, but no differences were observed among groups.

Hemodynamic findings in this study are summarized in Table 2. As noticed in our previous study, \( ^{6} \) systemic arterial blood pressure tended to decline in dogs treated with t-PA, heparin, and antiplatelet drugs during the course of the experiment. This was mainly due to some bleeding from the surgical wounds. As a consequence of this, the hematocrit in group 2 dogs decreased significantly from 36±4% at baseline to 30±3% at 90 minutes of reperfusion.

**Fibrinolytic Studies**

Fibrinogen levels significantly decreased after administration of t-PA to an average of 60±6% of preinfusion values in group 1, 62±5% in group 2, 61±5% in group 3, and 62±6% in group 4. Absolute levels of fibrinogen for each animal in the four groups before and after t-PA infusion are reported in Table 3.

**Discussion**

In the present study, we evaluated the role played by TXA\(_2\) and 5HT in mediating platelet activation during infusion of t-PA that may cause dynamic changes of the thrombus and affect the speed of thrombolysis. The major findings of our study are 1) TXA\(_2\) and 5HT appear to contribute to platelet
activation and inclusion in the thrombus; 2) combined treatment with SQ29548, a TXA2-receptor antagonist, and LY53857, a serotonin-receptor antagonist, markedly reduces the time and the total dose of t-PA necessary to achieve adequate reperfusion probably by preventing further platelet activation and deposition on the fibrin mesh already present in the thrombus during administration of t-PA; 3) administration of either antagonist alone does not enhance the thrombolytic effect of t-PA in this experimental model; and 4) treatment with SQ29548 and LY53857 prevents or delays (or both) reocclusion in this model.

It has been shown that arterial thrombi continue to grow by incorporation of new fibrin and platelets for up to 72 hours and that this growth is particularly fast during the first few hours.10,11 Thrombus growth that continues during lysis increases the total amount of thrombus to be lysed during thrombolytic treatment and thereby probably delays recanalization of the infarct-related artery and reduces the potential for myocardial salvage in patients with acute myocardial infarction. The balance between these two opposing processes may influence the clinical outcome of thrombolytic treatment. Heparin has been shown to prevent new fibrin formation and its incorporation into the thrombus, enhancing the thrombolytic effect of t-PA.12 Besides fibrin, however, one other major component of intracoronary thrombi is represented by platelet aggregates,28 and it has been demonstrated that activated platelets are able to bind fibrin avidly29 both through fibrinogen receptors associated with the glycoprotein IIb/IIIa complex and nonspecific binding.30

![Figure 3](image-url) **Figure 3.** Representative tracing of hemodynamic data obtained from a dog treated with heparin, t-PA and SQ29548 and LY53857 in combination. Panel A: Baseline measurements. Paper speed, 25 mm/sec. Panel B: After placement of the copper coil into the left anterior descending coronary artery (LAD) and thrombus formation. Paper speed, 25 mm/min. Panel C: t-PA administration caused lysis of the thrombus and restoration of blood flow that showed a stable and constant pattern throughout the experimental period. Paper speed, 25 mm/min. (See text for details.)

**Table 1.** Reocclusion Time and Activated Coagulation Time (ACT) in Dogs After Thrombolysis With t-PA

<table>
<thead>
<tr>
<th>Group</th>
<th>Reocclusion time (seconds)</th>
<th>Base (ACT) (seconds)</th>
<th>Reperfusion (ACT) (seconds)</th>
<th>90 minutes reperfusion or reocclusion (ACT) (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>17±5</td>
<td>97±6</td>
<td>207±15</td>
<td>199±12</td>
</tr>
<tr>
<td>Group 2</td>
<td>&gt;90 (9/9)*†</td>
<td>107±7</td>
<td>199±14</td>
<td>193±19</td>
</tr>
<tr>
<td>Group 3</td>
<td>31±29 (1/6)*</td>
<td>104±7</td>
<td>212±21</td>
<td>220±23</td>
</tr>
<tr>
<td>Group 4</td>
<td>27±31</td>
<td>100±13</td>
<td>227±24</td>
<td>204±19</td>
</tr>
</tbody>
</table>

Reocclusion time (minutes) was defined as the time elapsed between reperfusion and the occurrence of a persistent reocclusion.

*Numbers in parentheses indicate dogs in whom the treatment prevented reocclusion after thrombolysis.

ACT (seconds) was measured at baseline, at the moment of reperfusion, and after 90 minutes of reperfusion or at the moment of reocclusion. See text for details.

†p<0.005 vs. groups 1, 3, and 4 by Fisher's exact test.
This interaction could be responsible for further platelet deposition on the fibrin mesh in vivo after formation of the thrombus, and it may occur even if heparin is administered. In a recent study by Gold et al.\(^16\) blockade of the glycoprotein IIb/IIIa with a specific monoclonal antibody resulted in a significant enhancement of thrombolysis by t-PA and in prevention of reocclusion.

Platelets may be activated by several substances. In particular, studies from our laboratory with a canine model of concentrically stenosed and endothelially injured coronary arteries demonstrated the occurrence of a typical cyclic pattern of flow (CFVs) caused by alternating platelet aggregation and subsequent dislodgement of the thrombus and that TXA\(_2\) and 5HT are important mediators of CFVs.\(^17-20\) Blockade of TXA\(_2\) usually results in the elimination of CFVs.\(^17-20\)

It is interesting to note that in the present study neither SQ29548 nor LY53857 alone significantly enhanced thrombolysis and prevented CFVs and reocclusion, whereas the combination of the two antagonists was very effective in both enhancing thrombolysis and preventing CFVs and reocclusion. This is in contrast with previous studies from our laboratory employing a model of concentrically stenosed and endothelially injured canine coronary arteries\(^17-20\) in which either TXA\(_2\)- or 5HT-receptor blockade usually results in abolition of CFVs. However, it is possible that the presence of an intracoronary copper coil represents a stronger thrombogenic stimulus than a stenosed, endothelially injured coronary artery. Under these circumstances, one antagonist alone may not be sufficient to inhibit platelet activation. This is in agreement with a recent report that demonstrates a positive synergistic antiplatelet effect of serotonin- and TXA\(_2\)-receptor blockade in vivo.\(^31\)

Coronary thrombolysis is becoming an important approach in the treatment of patients with acute myocardial infarction because it is known that early reperfusion can reduce, under certain conditions, the extent of ischemic myocardium that will ultimately undergo necrosis.\(^32\) Several studies have provided evidence that the potential benefits of coronary thrombolysis may be significantly reduced or even offset by 1) an acute spontaneous reocclusion rate in 20–45% of the patients despite administration of heparin\(^1,2\) and 2) the time delay between the onset of myocardial ischemia and the lysis of the intracoronary thrombus.

In a recent study from our laboratory with the same model used in the present study, we demonstrated that reocclusion after thrombolysis by t-PA may occur despite the administration of heparin.\(^6\) We have also shown that this phenomenon is primarily caused by intracoronary platelet activation and that TXA\(_2\) and serotonin cooperatively mediate reocclusion.\(^6\) SQ29548 and LY53857, given at the moment of reperfusion, were able to prevent this

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**Table 2. Hemodynamic Variables in Dogs Before and After Thrombolysis With t-PA**

<table>
<thead>
<tr>
<th>Group 1 (n=5)</th>
<th>HR (beats/min)</th>
<th>AoM (mm Hg)</th>
<th>PHF (% of control)</th>
<th>MNF (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>143±11</td>
<td>127±5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>30 min occlusion</td>
<td>144±11</td>
<td>127±5</td>
<td>0.5±0.5</td>
<td>0.5±0.5</td>
</tr>
<tr>
<td>Beginning of reflow</td>
<td>141±11</td>
<td>121±6</td>
<td>88±9</td>
<td>99±11</td>
</tr>
<tr>
<td>Group 2 (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>133±17</td>
<td>122±11</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>30 min occlusion</td>
<td>129±17</td>
<td>122±11</td>
<td>0.7±0.6</td>
<td>0.7±0.6</td>
</tr>
<tr>
<td>Beginning of reflow</td>
<td>128±19</td>
<td>104±5</td>
<td>93±20</td>
<td>99±22</td>
</tr>
<tr>
<td>60 min reflow</td>
<td>128±19</td>
<td>90±9</td>
<td>74±9</td>
<td>78±11</td>
</tr>
<tr>
<td>90 min reflow</td>
<td>130±19</td>
<td>89±9</td>
<td>71±7</td>
<td>73±5</td>
</tr>
<tr>
<td>Group 3 (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>140±21</td>
<td>124±15</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>30 min occlusion</td>
<td>133±19</td>
<td>127±13</td>
<td>0.5±0.5</td>
<td>0.4±0.5</td>
</tr>
<tr>
<td>Beginning of reflow</td>
<td>134±19</td>
<td>122±11</td>
<td>87±17</td>
<td>96±22</td>
</tr>
<tr>
<td>60 min reflow</td>
<td>137</td>
<td>93</td>
<td>77</td>
<td>81</td>
</tr>
<tr>
<td>90 min reflow</td>
<td>140</td>
<td>89</td>
<td>71</td>
<td>76</td>
</tr>
<tr>
<td>Group 4 (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>142±20</td>
<td>125±20</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>30 min occlusion</td>
<td>140±20</td>
<td>120±18</td>
<td>0.3±0.4</td>
<td>0.2±0.3</td>
</tr>
<tr>
<td>Beginning of reflow</td>
<td>137±20</td>
<td>127±15</td>
<td>90±23</td>
<td>94±23</td>
</tr>
<tr>
<td>60 min reflow</td>
<td>129</td>
<td>97</td>
<td>81</td>
<td>84</td>
</tr>
<tr>
<td>90 min reflow</td>
<td>134</td>
<td>88</td>
<td>79</td>
<td>81</td>
</tr>
</tbody>
</table>

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.
phenomenon. In the present study, we provide evidence that TXA₂ and 5HT contribute to dynamic changes of the thrombus during thrombolysis. Simultaneous administration of SQ and LY during infusion of t-PA markedly enhanced thrombolysis and reduces the dose of t-PA necessary to achieve reperfusion.

The doses of SQ29548 and LY53857 we used should have achieved a maximal blockade of their respective receptors. Fitzgerald et al have demonstrated that SQ29548 at the dose of 0.2 mg/kg bolus followed by a continuous infusion of 0.2 mg/kg/hr completely inhibits arachidonic acid or U46619 (a TXA₂ mimetic) induced aggregation in ADP-primed canine platelets. We cannot absolutely exclude the possibility that the interaction between SQ29548 and LY53857 observed in the present study may be pharmacokinetic and not pharmacodynamic (i.e., one antagonist had increased the plasma concentration of the other). However, this possibility seems very unlikely because LY53857 at half of the dose used in the present study and SQ29548 at a dose comparable to that used in the present study elicited a complete blockade of their respective receptors. Therefore, even if one antagonist had increased the plasma concentration of the other, it is very unlikely that this would have led to a further increase in the degree of reperfusion. Indeed, our hypothesis that simultaneous blockade of TXA₂-PGH₂ and 5HT₂ receptors results in a greater antiplatelet effect than either intervention alone is strongly supported by several studies. It is, in fact, known that one platelet agonist added to platelet-rich plasma in a concentration too low to cause aggregation enhances the response and leads to induction of aggregation upon addition of another platelet agonist in similar subthreshold concentration. This phenomenon had been demonstrated in vitro for several pairs of agonists and in vivo for TXA₂ and 5HT. It has been suggested that this synergism between agonists results from an increase in the concentration of cytoplasmic Ca²⁺, which acts on a second messenger. In addition, it has been reported that the combination of TXA₂-PGH₂- and 5HT₂-receptor blockade resulted in a synergistic inhibition of in vitro human platelet aggregation and in a synergistic prolongation of the rat tail bleeding time, thus indicating a greater antithrombotic activity when both receptors are blocked simultaneously.

In the present study, we used SQ29548 and LY53857 as a TXA₂-PGH₂- and a 5HT₂-receptor antagonist, respectively, because of a high specificity for their respective receptors. Ogletree et al have demonstrated that SQ29548 is highly selective for TXA₂-PGH₂ receptors with respect to a wide group of agonists, including serotonin. Furthermore, Cohen et al have demonstrated the specificity of LY53857 in blocking 5HT₂ receptors. In addition, in two recent papers from our laboratory, we have shown that SQ29548 does not inhibit serotonin-induced aggregation in epinephrine-primed canine platelets and that LY53857 does not alter U46619-induced aggregation in epinephrine-primed canine platelets.

In the present study, dogs treated with either antagonist alone were not protected against reoclusion after stopping t-PA. This might have been the result of a decrease in the plasma concentration of the drugs below the effective level. Although we cannot absolutely exclude this possibility because we did not measure the plasma half-lives of the two antagonists, it seems quite unlikely because in our previous study, reoclusion occurred in the vast majority of the dogs treated with either antagonist alone despite the fact that the drugs were given at the moment of reperfusion. This finding suggests
that reocclusion occurs in most of the animals receiving either antagonist alone even in the presence of plasma levels of the drugs sufficient to achieve blockade of their respective receptors. Furthermore, in a previous study from our laboratory, we have demonstrated that cyclic flow variations in endothelially injured, stenosed canine coronary arteries are completely abolished for 2.9±0.8 hours after administration of a single bolus of SQ29548 of 0.1 mg/kg, indicating that the antplatelet effect of SQ29548 is present well after the time elapsed, in the present study, between administration of the drugs and occurrence of reocclusion.

Conclusions
The clinical efficacy of coronary thrombolysis is clearly dependent on the rapidity by which reperfusion can be achieved after the onset of myocardial ischemia and the rate and frequency of early reoclusion. We have demonstrated that a significant enhancement of thrombolysis can be achieved by simultaneous administration of TXA2- and 5HT-receptor antagonists and that this treatment is also able to prevent early reocclusion.

It is always difficult to extrapolate data from the experimental setting to clinical practice and the present study has several potential limitations, such as the presence of an intracoronary copper coil, which is a rather "unphysiologic" model of thrombosis, and the fact that the coil was left in place after lysis of the thrombus. However, the observed differences in the present study were substantial. The marked effect of the combination of TXA2- and 5HT-receptor antagonists on the enhancement of thrombolysis and in the prevention of acute coronary reocclusion provides new insights into the pathophysiology of coronary reocclusion after thrombolysis and may have important implications for future thrombolytic therapy in selected patients with acute myocardial infarction.

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**KEY WORDS** • thromboxane A2 • serotonin • thrombolysis • platelet activation
Simultaneous administration of thromboxane A2- and serotonin S2-receptor antagonists markedly enhances thrombolysis and prevents or delays reocclusion after tissue-type plasminogen activator in a canine model of coronary thrombosis.

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