Platelet and Vascular Function During Coronary Thrombolysis With Tissue-Type Plasminogen Activator

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Platelet activation may limit the response to tissue-type plasminogen activator (t-PA) during coronary thrombolysis in humans. As an index of platelet activation, we assessed thromboxane A2 biosynthesis during coronary thrombolysis with intravenous t-PA in patients with acute myocardial infarction. Urinary 2,3-dinor-thromboxane B2, a metabolite of thromboxane A2, was increased to a peak of 3,327±511 pg/mg creatinine (n=12) following administration of intravenous t-PA and remained elevated for 48 hours. This increase was abolished by pretreatment with aspirin 325 mg orally (n=6), indicating de novo biosynthesis of thromboxane A2 rather than washout of preformed metabolites during reperfusion. Prostacyclin (PGI2) biosynthesis, determined by excretion of 2,3-dinor-6-keto-PGF1α, also increased after t-PA administration. However, this increase was less pronounced in patients who reperfused (28±3.3 ng/hr/mg creatinine) than in patients who failed to reperfuse (118±30 ng/hr/mg creatinine, p<0.05). These data provide evidence of platelet activation during coronary thrombolysis with t-PA. In patients who reperfused, the reduction in PGI2 biosynthesis may be a marker of reperfusion injury to the vasculature and may further amplify platelet activation. (Circulation 1989;80:1718-1725)

Platelet activation plays a major role in limiting the response to tissue-type plasminogen activator (t-PA) in experimental models of coronary thrombosis.1-4 In particular, platelets delay reperfusion and induce reocclusion.1,4 Platelet activation is also increased in patients with acute myocardial infarction treated with intravenous streptokinase,5 and antiplatelet therapy enhances the response to streptokinase, accelerating reperfusion6 and further reducing mortality.7 However, streptokinase directly activates human platelets.8 In contrast, t-PA inhibits platelet function in vitro.8 Although t-PA has been reported to increase platelet aggregation ex vivo,9 this increase may reflect an artifact due to plasminogen activation enhanced by fibrin formed during sampling10 and a proaggregatory response to plasmin.11 Whether platelet activation also occurs in vivo during coronary thrombolysis induced by t-PA is unknown. In addition, there is little information on what regulates platelet activation in this setting.

In the present study we examined thromboxane (Tx) A2 biosynthesis as an index of platelet activation during coronary thrombolysis with t-PA in humans. TxA2, the major cyclo-oxygenase product of arachidonic acid derived from platelets, is formed by platelets on activation and amplifies the platelet aggregation and release response to many agonists.12 We also determined biosynthesis of prostacyclin (PGI2), a major cyclo-oxygenase product of arachidonic acid in endothelium and a potent platelet inhibitor.13 Formation of these eicosanoids is increased in conditions associated with platelet activation, including unstable angina, acute myocardial infarction,14 and severe peripheral vascular disease,15 which is consistent with the hypothesis that they regulate platelet function in vivo.

Methods

Study Protocol

The study group consisted of patients admitted with chest pain of cardiac origin of less than 4 hours...
duration who had electrocardiographic evidence of an acute myocardial infarction. Patients were excluded if they had taken aspirin or any other cyclo-oxygenase inhibitor in the 10 days before admission. All patients gave written, informed consent. t-PA (Genentech, South San Francisco, California) was administered intravenously as a 7-mg bolus followed by a continuous infusion of 53 mg over the first hour, 20 mg over the second hour, and then 10 mg/hr over the subsequent 2 hours.

Biosynthesis of TxA2 and PGI2 was determined as urinary excretion of their enzymatic metabolites, 2,3-dinor-TxB2 and 2,3-dinor-6-keto-prostaglandin (PG) F1α, respectively. Urine was collected over 4 hours after the administration of t-PA and every 6 hours thereafter for 48 hours. Urine was also collected for 24 hours on day 5. In addition, where possible, a spot urine sample was obtained immediately before t-PA administration. Plasma creatine phosphokinase (CPK) was measured at 6-hour intervals over the first 24 hours and then every 12 hours. Fibrinogen was determined before initiation of t-PA infusion and 2 hours after its discontinuation. Platelet counts were determined before t-PA and also in the 24 hours after t-PA administration.

In addition to t-PA, heparin 1,000 units/hr was administered intravenously 90 minutes after initiation of the t-PA infusion and adjusted to maintain the cephalin kaolin time at twice control. All patients received prophylactic lidocaine given as a bolus dose of 3 mg/kg followed by an infusion of 1.75 mg/kg/hr, sublingual nitrates, and morphine 3–9 mg i.v. Although heparin has been shown to activate platelets in vitro, we and others have demonstrated that heparin does not alter TxA2 biosynthesis in humans.5–16 It has also been reported that nitrates induce PGI2 formation by isolated vascular tissue.17 However, we have demonstrated that nitrates do not alter PGI2 biosynthesis in patients with coronary artery disease.18 Cardiac catheterization was performed if clinically indicated after completion of the 48-hour period of urine collection. This delay was necessary as an artifactual increase in eicosanoid biosynthesis is induced by tissue trauma and platelet activation during the procedure.19 Patients were considered to have reperfused if the vessel was patent at the time of coronary catheterization or if the time to peak CPK was less than or equal to 12 hours after the initiation of t-PA.

Urine was also obtained from a group of patients with unstable angina admitted over the same period. Unstable angina was defined as at least one episode of chest pain, typical of ischemia, occurring at rest and lasting for 15 minutes or more. All patients were admitted into the study within 24 hours of their last episode of chest pain, and all were demonstrated to have coronary artery disease on subsequent coronary angiography. These patients received intravenous nitrates and heparin as described for the patients receiving t-PA. In addition, data from patients receiving thrombolytic therapy were compared with previously published data from a group of patients with acute myocardial infarction who did not receive thrombolytic therapy.14 These patients had been well characterized and had been studied according to the same protocol, other than not receiving t-PA. Distribution of the infarcts and clinical course were similar in the two groups.

Aspirin-Treated Patients

To address the origin of the thromboxane metabolites, 325 mg aspirin were administered orally at the time of admission immediately before the administration of t-PA in six additional patients. The clinical features, site of infarction, and peak plasma CPK were similar to those treated with t-PA alone.

Biochemical Studies

Urine was analyzed for 2,3-dinor-TxB2 and 2,3-dinor-6-keto-PGF1α by gas chromatography and negative-ion chemical ionization mass spectrometry, as previously described.20,21 For 2,3-dinor-TxB2, 1 ng/ml tetradeuterated 2,3-dinor-TxB2 was added to 3 ml urine. After initial formation of the methoxime derivative, the samples were selectively extracted by passage through bonded-phase, phenylboronic acid columns and then subjected to thin-layer chromatography (TLC). After the formation of the pentafluorobenzyl ester and further TLC, the trimethylsilyl ether derivative was formed. Quantitative analysis was performed on a NERMAG 10-10C mass spectrometer operating in the negative-ion mode coupled with a Varian Vista 6000 gas chromatograph, monitoring m/z 586 for endogenous 2,3-dinor-TxB2 and m/z 590 for the tetradeuterated internal standard.

2,3-Dinor-6-keto-PGF1α was determined in a 3-ml urine sample after the addition of 1 ng/ml tetradeuterated 2,3-dinor-6-keto-PGF1α as internal standard. After selective extraction under acidic and alkaline conditions, as previously described,21 the methoxime derivative was formed. After further purification by TLC, the pentafluorobenzyl ester was formed and the sample finally derivatized to the trimethylsilyl ether. Quantitation was performed as for 2,3-dinor-TxB2 with selective ion monitoring at m/z 586 and m/z 590 for the endogenous 2,3-dinor-6-keto-PGF1α and the tetradeuterated internal standard, respectively.

Fibrinogen was determined as clottable protein by the Clauss method.22 Platelet counts were performed by the Coulter technique.

Statistical Analysis

Values are given as mean±SEM. Excretion of metabolites and plasma CPK were integrated (area under the concentration-time curve) over the first 48 hours as an index of total excretion. Metabolite excretion was analyzed within patients and between groups by Kruskal-Wallis analysis of variance or rank-sum test where appropriate. Rank-Spearman cor-
TABLE 1. Clinical Features and Angiographic Findings in Patients Who Received t-PA With or Without Previous Aspirin

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Infarct site</th>
<th>Onset of symptoms to t-PA infusion (min)</th>
<th>Peak CPK (units/ml)</th>
<th>Time to peak CPK (hr)</th>
<th>Angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>Anterior</td>
<td>205</td>
<td>4,580</td>
<td>6</td>
<td>LAD 80%</td>
</tr>
<tr>
<td>63</td>
<td>Anterior</td>
<td>120</td>
<td>1,750</td>
<td>6</td>
<td>LAD 60%</td>
</tr>
<tr>
<td>52</td>
<td>Anterior</td>
<td>240</td>
<td>2,170</td>
<td>6</td>
<td>LAD 80%</td>
</tr>
<tr>
<td>67</td>
<td>Anterior</td>
<td>133</td>
<td>4,590</td>
<td>6</td>
<td>None</td>
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<tr>
<td>57</td>
<td>Anterior</td>
<td>120</td>
<td>6,600</td>
<td>6</td>
<td>LAD 80%</td>
</tr>
<tr>
<td>44</td>
<td>Anterolateral</td>
<td>210</td>
<td>2,970</td>
<td>6</td>
<td>LAD 90%</td>
</tr>
<tr>
<td>34</td>
<td>Posteroinferior</td>
<td>120</td>
<td>2,162</td>
<td>7</td>
<td>RCA 70%</td>
</tr>
<tr>
<td>54</td>
<td>Anterolateral</td>
<td>55</td>
<td>3,460</td>
<td>18</td>
<td>RCA 60%</td>
</tr>
<tr>
<td>54</td>
<td>Posteroinferior</td>
<td>130</td>
<td>1,050</td>
<td>18</td>
<td>None</td>
</tr>
<tr>
<td>62</td>
<td>Anterolateral</td>
<td>130</td>
<td>2,300</td>
<td>18</td>
<td>None</td>
</tr>
<tr>
<td>53</td>
<td>Anterolateral</td>
<td>150</td>
<td>4,150</td>
<td>13</td>
<td>None</td>
</tr>
<tr>
<td>70</td>
<td>Anterolateral</td>
<td>145</td>
<td>2,571</td>
<td>24</td>
<td>LAD occluded</td>
</tr>
</tbody>
</table>

Patients who received aspirin* immediately before t-PA

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Infarct site</th>
<th>Onset of symptoms to t-PA infusion (min)</th>
<th>Peak CPK (units/ml)</th>
<th>Time to peak CPK (hr)</th>
<th>Angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>Anterior</td>
<td>40</td>
<td>790</td>
<td>7</td>
<td>LAD 90%</td>
</tr>
<tr>
<td>67</td>
<td>Lateral</td>
<td>80</td>
<td>1,792</td>
<td>6</td>
<td>OM 90%</td>
</tr>
<tr>
<td>40</td>
<td>Inferior</td>
<td>105</td>
<td>2,327</td>
<td>4</td>
<td>RCA 50%</td>
</tr>
<tr>
<td>56</td>
<td>Anterior</td>
<td>170</td>
<td>3,100</td>
<td>6</td>
<td>None</td>
</tr>
<tr>
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<td>Anterior</td>
<td>40</td>
<td>2,009</td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td>58</td>
<td>Inferior</td>
<td>125</td>
<td>1,120</td>
<td>18</td>
<td>RCA occluded</td>
</tr>
</tbody>
</table>

*325 mg orally.
CPK, creatine phosphokinase; LAD, left anterior descending coronary artery; RCA, right coronary artery; OM, obtuse marginal coronary artery.

relations were performed between plasma CPK and urinary metabolites for the different groups. These analyses are nonparametric and, therefore, make no assumptions about the distribution of the data.23

Results

Twelve patients (11 men, one woman) received t-PA alone (Table 1). An additional six patients (five men, one woman) received aspirin immediately before t-PA. Medications before admission included calcium channel blockers in two and β-blockers in three patients. One patient received nifedipine 10 mg s.l. on admission. t-PA was administered 128±13 minutes (range, 40–240 minutes) from the onset of chest pain. Eight of the patients who did not receive aspirin had an early rise in plasma CPK with a peak CPK at 6.1±0.1 hours from the onset of t-PA infusion. Coronary arteriography was performed in seven of these patients, and all had patent infarct-related vessels. In four patients peak CPK occurred at 18.3±2.3 hours. Two of these patients had coronary angiography, and both were found to have an occluded infarct-related artery. Five of the six patients treated with aspirin had an early rise in plasma CPK (Table 1). Three of these patients underwent cardiac catheterization, and all had patent infarct-related coronary arteries. The sixth aspirin-treated patient had a late rise in plasma CPK and had an occluded vessel at angiography.

Fibrinogen decreased from 297±24 mg/dl on admission to 160±14 mg/dl 2 hours after discontinuation of t-PA (p<0.01). The platelet count declined from 290±14×10^3/μl to 248±12×10^3/μl after t-PA (p<0.05). After t-PA one patient developed a groin hematoma and another marked hematuria. A third developed hypotension requiring treatment with dobutamine.

Urinary 2,3-dinor-TxB₂ was markedly elevated in the first urine collection after t-PA administration (2,584±463 pg/mg creatinine; normal, <350 pg/mg creatinine) and rose to a peak of 3,327±511 pg/mg creatinine within 10 hours of t-PA (Figure 1). Sub-
sequently, the levels fell but remained above normal over the first 48 hours. By day 5, urinary 2,3-dinor-TxB₂ had returned to normal. In contrast, peak urinary 2,3-dinor-TxB₂ was 638±107 pg/mg creatinine in patients with unstable angina (p<0.005 vs. peak after t-PA; n=14), a condition in which TxA₂ has been shown to be of pathophysiologic importance.²⁴⁻²⁶ Furthermore, urinary 2,3-dinor-TxB₂ was 537±102 pg/mg creatinine over the first 6 hours after admission in the previously described group of patients with acute myocardial infarction not treated with a thrombolytic agent and showed no further increase.¹⁴ Two patients were able to provide a urine sample before t-PA administration. Urinary 2,3-dinor-TxB₂ was 315 and 1,014 pg/mg creatinine before t-PA, similar to patients not receiving a thrombolytic agent. Both patients showed a marked increase in metabolite excretion after administration of t-PA (Figure 2). When the patients who reperfused were compared with those who remained occluded, analysis of variance demonstrated an increased excretion of 2,3-dinor-TxB₂ during the second urine collection after t-PA administration (p<0.05); metabolite excretion did not differ significantly between the two groups at any other period. The increase in urinary 2,3-dinor-TxB₂ induced by t-PA was markedly suppressed in all patients treated with aspirin (Figure 1).

Excretion of 2,3-dinor-6-keto-PGF₁α (normal, <220 pg/mg creatinine) also increased in patients treated with t-PA alone (Figures 2 and 3). However, this increase was less in patients who reperfused. Total excretion of 2,3-dinor-6-keto-PGF₁α over the first 48 hours was 28±3.3 ng/hr/mg creatinine (n=8) in patients who reperfused compared with 118±30 ng/hr/mg creatinine (n=4; p<0.05) in patients who failed to reperfuse. This difference was all the more striking when 2,3-dinor-6-keto-PGF₁α excretion was related to plasma CPK. In patients who failed to reperfuse, there was a positive correlation between total excretion of this metabolite and plasma CPK (r=0.99, n=4, p<0.05). This had also been demonstrated previously in patients not receiving thrombolytic therapy (r=0.93, n=12; p<0.001).¹⁴ Indeed, the data for the two groups were superimposable (Figure 4). In contrast, there was no correlation between excretion of 2,3-dinor-6-keto-PGF₁α and plasma CPK in patients who reperfused, as shown in Figure 4 (r=-0.52, n=8, NS).

**Discussion**

The response to coronary thrombolysis in humans is limited by delayed or failed reperfusion and by acute reocclusion, the mechanisms of which are poorly understood.²⁷ Although clinical studies suggest that these factors reflect ongoing thrombosis,²⁸ routine anticoagulation with heparin appears to have little effect.²⁹ In this study we demonstrated an increase in the biosynthesis of TxA₂ after t-PA administration in patients with an acute myocardial infarction. This increase far exceeded that seen in patients with unstable angina, a condition in which TxA₂ has been shown to play a pathophysiologic role.²⁴⁻²⁶ In addition to an increase in the formation of proaggregatory TxA₂, biosynthesis of PGI₂, a potent platelet inhibitor, was depressed in patients who reperfused after the administration of t-PA. These data provide evidence of enhanced platelet activation and an alteration in factors that regulate platelet activity during coronary thrombolysis with t-PA.

As an index of platelet activation in these studies, we assessed TxA₂ biosynthesis. TxA₂ biosynthesis is increased in conditions associated with platelet activation, and its measurement as an index of platelet activation avoids the artifacts inherent in ex vivo platelet studies.³⁰ This may be particularly important in the setting of thrombolytic therapy where ex vivo plasminogen activation may alter platelet function. Because TxA₂ itself is unstable and cannot be measured directly, we determined...
the excretion of 2,3-dinor-TxB₂, an enzymatic metabolite of TxA₂ that is rapidly cleared by the kidney. Measurement of 2,3-dinor-TxB₂ in urine avoids many of the artifacts inherent in the measurement of the nonenzymatic product, TxB₂, in plasma and is an index of systemic TxA₂ formation.

In this study urinary excretion of 2,3-dinor-TxB₂ increased over the first 48 hours after the administration of t-PA and returned to normal by day 5. To assess the possibility that the increase in metabolite excretion reflected washout of preformed, inactive TxB₂ from the lysed thrombus and its metabolism to 2,3-dinor-TxB₂, we examined the effect of pretreating patients with aspirin. In a dose that markedly inhibits platelet cyclo-oxygenase, aspirin abolished the increase in 2,3-dinor-TxB₂ excretion. It is unlikely that differences in TxA₂ biosynthesis between the two groups were due to clinical factors. These patients were comparable with respect to infarct size and distribution, and there was no correlation in any of the groups between plasma CPK and excretion of 2,3-dinor-TxB₂. Therefore, TxA₂ biosynthesis increases during coronary thrombolysis with t-PA and returns to normal by day 5 after coronary thrombolysis. TxA₂ is formed by many tissues. However, selective inhibition of platelet cyclo-oxygenase in a variety of settings suggests that 2,3-dinor-TxB₂ is largely derived from platelet TxA₂. In keeping with this possibility, a single dose of aspirin abolished the increase in excretion of this metabolite in patients receiving t-PA. Thus, the increase in TxA₂ biosynthesis during coronary thrombolysis is consistent with platelet activation although other tissue sources cannot be excluded.

For ethical reasons, it was not possible to study a comparable group with untreated acute myocardial infarction at the same time. Therefore, we compared the patients receiving t-PA with a group of patients with acute myocardial infarction who did not receive thrombolytic therapy. TxA₂ biosynthesis in patients receiving thrombolytic therapy greatly exceeded that seen in patients with unstable angina, a condition characterized by coronary thrombosis in which studies with aspirin have confirmed the functional significance of TxA₂. TxA₂ biosynthesis was also greater in the patients receiving t-PA than we have previously reported in acute myocardial infarction. In patients with acute myocardial infarction who did not receive thrombolytic therapy, urinary 2,3-dinor-TxB₂ was only modestly elevated on admission and showed no further increase during a comparable 48-hour time period. In contrast, in two patients in whom urine was obtained before t-PA administration, 2,3-dinor-TxB₂ excretion, similar on admission to those of untreated patients, increased after t-PA. While comparisons with a historical group and with unstable angina must be viewed with caution and the latter group does not serve as a control for the effect of t-PA, they support the hypothesis that TxA₂ biosynthesis increases after the administration of t-PA to an extent that may be functionally important.

The increase in TxA₂ formation provides evidence that platelet activity is increased during coronary thrombolysis with t-PA. Platelet activation delays reperfusion and is a major mechanism of acute reclosure in animal models of coronary thrombosis. There is also evidence that platelet activation delays reperfusion in response to intracoronary streptokinase in humans. Therefore, the increase in platelet activation during coronary thrombolysis with t-PA may limit the response to this therapy. In addition to indicating platelet activation, the increase in TxA₂ formation may of itself be functionally important. We have demonstrated a marked increase in TxA₂ biosynthesis in patients with acute myocardial infarction treated with intravenous streptokinase. More recently, in the ISIS-II trial, aspirin has been shown to induce a further reduction in the mortality compared with streptokinase alone in patients with an acute myocardial
infarction.\textsuperscript{7} These findings suggest that TxA\textsubscript{2} can impair the clinical response to thrombolytic therapy. TxA\textsubscript{2} is a potent platelet agonist and may be a mediator of platelet activation during coronary thrombosis, as demonstrated in animal models.\textsuperscript{2,4} TxA\textsubscript{2} may also induce vasospasm during coronary thrombosis\textsuperscript{36,37} and may be a determinant of infarct size in the setting of reperfusion.\textsuperscript{38,39}

This study also demonstrates that patients who reperfuse with t-PA fail to show the normal infarct-related rise in PGI\textsubscript{2} biosynthesis, as determined by excretion of its enzymatic metabolite, 2,3-dinor-6-keto-PGF\textsubscript{1\alpha}. In patients with acute myocardial infarction who are not receiving a thrombolytic agent, PGI\textsubscript{2} formation rose over the 48 hours after admission and gradually declined toward normal by day 5.\textsuperscript{5,14,40} This activity correlated closely with plasma CPK level, and striking increases occurred in patients with large myocardial infarctions, findings that suggest that the increase in PGI\textsubscript{2} biosynthesis derives from the heart.\textsuperscript{14,41} The cellular origin is uncertain, but it is probably vascular endothelium because isolated myocardial cells have no prostaglandin synthase activity and endothelial cells are the major source of PGI\textsubscript{2} in myocardial tissue.\textsuperscript{42} Furthermore, injured myocytes release arachidonic acid,\textsuperscript{43} which may be metabolized by other cells to form prostaglandins including PGI\textsubscript{2}.\textsuperscript{44}

Although the number of patients was small, a similar increase in PGI\textsubscript{2} was seen in patients who failed to reperfuse after t-PA administration. In contrast, the increase in PGI\textsubscript{2} biosynthesis was blunted in patients who reperfused, and it did not correlate with plasma CPK. The normal prolonged increase in PGI\textsubscript{2} biosynthesis also fails to occur following reperfusion with streptokinase.\textsuperscript{5} The mechanism of the reduction in PGI\textsubscript{2} biosynthesis after coronary reperfusion is uncertain. Reperfusion may reduce infarct size and, as a consequence, PGI\textsubscript{2} biosynthesis. However, because CPK release correlates with infarct size, even in the setting of reperfusion,\textsuperscript{45–47} there would continue to be a relation between plasma CPK and PGI\textsubscript{2} formation. In contrast, no correlation was found between these variables after reperfusion with t-PA. An alternative explanation is that the decrease in PGI\textsubscript{2} formation reflects reperfusion injury of the coronary artery. In canine experiments, reperfusion of the ischemic vascular bed results in histologic and functional evidence of endothelial injury.\textsuperscript{48} Recent studies in vitro suggest that this may be mediated by oxygen free radicals generated during reperfusion and reoxygenation of the ischemic endothelium.\textsuperscript{49,50} Thus, the failure to increase PGI\textsubscript{2} biosynthesis after reperfusion with t-PA may be due to reperfusion injury of the vascular endothelium in the area of infarction.

What induces platelet activation during coronary thrombosis with t-PA is uncertain. Experimental studies suggest that the coronary bed is the major site of platelet activation in this setting. Platelets may be activated at the site of thrombolysis by plasmin\textsuperscript{10,51,52} or by the continued formation of thrombin.\textsuperscript{53,54} This process may explain the increased TxA\textsubscript{2} formation seen even in patients who fail to reperfuse. Further activation may occur with exposure of platelets to the injured vascular bed after reperfusion.\textsuperscript{4,55} Although it is also possible that platelet activation occurs systemically, as a reflection of plasminemia, this seems unlikely as platelet activation takes place only at high concentrations of plasmin (>1 CU/ml),\textsuperscript{51} which are not achieved in vivo. Thus, plasma α\textsubscript{2}-antiplasmin, which forms 1:1 stoichiometric complexes with plasmin,\textsuperscript{56} decreases by only 25% after t-PA administration in humans.\textsuperscript{57} Therefore, the systemic concentration of plasmin in vivo is unlikely to be sufficient to induce platelet activation. On the contrary, low plasmin concentrations may inhibit platelet function.\textsuperscript{58} Whatever the mechanism of platelet activation during coronary thrombolysis, the reduction in PGI\textsubscript{2} biosynthesis in patients who reperfuse may further amplify platelet activation in this setting. PGI\textsubscript{2} formation is increased in conditions associated with platelet activation,\textsuperscript{10,59,60} and biologically effective concentrations are formed at the site of vascular injury in humans.\textsuperscript{61} This increase suggests that PGI\textsubscript{2} may regulate platelet activity at the platelet-vessel wall interface. Inhibition of vascular cyclo-oxygenase increases platelet deposition in vivo, which is consistent with this hypothesis.\textsuperscript{62,63} Thus, the loss of PGI\textsubscript{2} formation may allow platelet adhesion and subsequent aggregation to occur in the reperfused vascular bed.

While these studies provide evidence for platelet activation during coronary thrombolysis, the combined use of platelet inhibition and thrombolytic agents must be viewed with caution. Preliminary data suggest that the risk of hemorrhage during coronary thrombolysis is increased in patients with prolonged bleeding times.\textsuperscript{64} While the ISIS-II trial showed no increased risk of bleeding when aspirin was combined with streptokinase, more potent platelet inhibitors may increase this risk.

In conclusion, platelet activation is increased during coronary thrombolysis with t-PA in humans. These data are consistent with studies in experimental models demonstrating that platelets limit the thrombolytic response to t-PA. A possible mediator of platelet activation during coronary thrombolysis with t-PA is TxA\textsubscript{2} because biosynthesis of this eicosanoid is increased. In addition, these data demonstrate a reduction in PGI\textsubscript{2} biosynthesis in patients who reperfuse. This may be a noninvasive marker of reperfusion injury in humans and may amplify further platelet activation during coronary thrombolysis. These data support the further evaluation of antiplatelet therapy and TxA\textsubscript{2} inhibition during coronary thrombolysis with t-PA.

Acknowledgments

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References

1. Schumacher WA, Lee EC, Luchessi BR: Augmentation of streptokinase-induced thrombolysis by heparin and prosta-
agonists on intracoronary platelet deposition in dogs with experimentally stenosed coronary arteries. Circulation
1988;78:701–711
BS: Monoclonal antibody against the platelet glycoprotein (Gp) Ib/IIa receptor prevents coronary artery reocclusion
4. Fitzgerald DJ, Wright F, FitzGerald GA: Increased throm-
boxane biosynthesis during coronary thrombolysis: Evidence that TXA2 modulates the response to tissue-type plasminogen
5. Fitzgerald DJ, Catella F, Roy L, FitzGerald GA: Marked platelet activation in vivo after intravenous streptokinase
in patients with acute myocardial infarction. Circulation
1988;77:142–150
thrombolysis and left ventricular function in acute myocardial
infarction (abstract). J Am Coll Cardiol 1988;11:104A
7. ISIS-II (Second International Study Group of Infarct Sur-
vival) Collaborative Group: Randomized trial of intravenous
streptokinase, oral aspirin, both or neither in 17,187 cases of
suspected acute myocardial infarction: ISIS-II. Lancet
1988;2:349–360
8. Loscalzo J, Vaughan DE: Tissue plasminogen activator
promotes platelet disaggregation in plasma. J Clin Invest
1987;79:1749–1755
plasminogen activator and streptokinase induce platelet hyper-
vation but is not an aggregating agent. Am J Physiol 1988;
255:H1276–H1288
11. Niewiarowski S, Senyi AF, Gillies P: Plasmin-induced plate-
etlet aggregation and platelet release reaction: Effects on
platelets. Adv Prostaglandin Thromboxane Leukotriene Res
1982;10:15–57
walls generate from prostaglandin endoperoxides a sub-
stance (prostaglandin X) which relaxes strips of mesenteric and
coeiac arteries and inhibits platelet aggregation. Prosta-
glandins 1976;12:897–913
1986;315:983–989
15. Reilly IAG, Doran JB, Smith B, FitzGerald GA: Increased
thromboxane biosynthesis in a human preparation of platelet
activation: Biochemical and functional consequences of selec-
tive inhibition of thromboxane synthase. Circulation
1986;73:1300–1309
16. Ljungberg B, Beving H, Egberg N, Johnsson H, Vestergqvist
O: Immediate effects of heparin and LMW heparin on some
platelet and endothelial derived factors. Thromb Res
1988;51:209–217
17. Levin RJ, Jaffé EA, Weksler BB, Tack-Goldman K: Nitro-
glycerin stimulates synthesis of prostacyclin by cultured
18. Fitzgerald DJ, Roy L, Robertson RM, FitzGerald GA: The
effects of organic nitrates on prostacyclin biosynthesis and
platelet function in humans. Circulation 1984;70:297–302
19. Roy L, Knapp HR, Robertson RM, FitzGerald GA: Endoge-
rous biosynthesis of prostacyclin during cardiac catheterization
20. Lawson JA, Brash AR, Doran J, FitzGerald GA: Measure-
ment of urinary 2,3-dinor thromboxane B2 and thromboxane
B2 using bonded-phase phenylboronic acid columns and
capillary gas chromatography-negative-ion chemical ioniza-
tion mass spectrometry. Anal Biochem 1985;150:463–470
21. FitzGerald GA, Brash AR, Falardeau P, Oates JA: Esti-
imated rate of prostacyclin secretion into the circulation
22. Clauss A: Rapid physiological coagulation method for de-
23. Siegel S: Nonparametric Statistics for the Behavioral Sci-
24. Lewis HD, Davis JW, Archibald DG, Steinke WE, Smith-
erman TC, Doherty JE, Schnaper HW, LeWinter MM, Linares E, Pouget JM, Sabharwal SC, Chesler E, DeMots
H: Protective effects of aspirin against acute myocardial
1983;309:396–403
DA, Jablonsky G, Kostuk WJ, Melendez LJ, Myers
MG, Sackett DL, Sealey DL, Tanser PH: Aspirin, sulfnylpy-
razone, or both in unstable angina: Results of a Canadian
F, Pelletier E, Juneau M, Stasiak J, DeGuise P, Pelletier GB,
Rinzler D, Waters D: Aspirin, heparin, or both to treat acute
28. Johns JA, Gold HK, Leinbach RC, Yasuda T, Gimple LW,
Werner W, Finkelstein D, Newell J, Ziskind AA, Collen
D: Prevention of coronary artery reocclusion and reduction in
late coronary artery stenosis after thrombolytic therapy in
patients with acute myocardial infarction: A randomized
study of maintenance infusion of recombinant human tissue-
29. Topol EJ, George BS, Kereiakes DJ, Candela RJ, Abbotts-
worth CM, Aronson L, O’Neill WW, Stack RS, Calif
RM: A multicenter, randomized, controlled trial of intravenous
tissue plasminogen activator and early intravenous heparin in
acute myocardial infarction (abstract). J Am Coll Cardiol
1988;11:232A
30. FitzGerald GA, Persson AK, Patrono C: Analysis of pro-
tacyclin and thromboxane biosynthesis in cardiovascular
lived enzymatic metabolites of thromboxane B2 in the human
32. Catella F, FitzGerald GA: Paired analysis of urinary throm-
47:647–656
33. Patrignani P, Filabozzi P, Patrono C: Selective cumulative
inhibition of platelet thromboxane production by low dose
34. Fitzgerald DJ, Mayo G, Catella F, Entman SS, FitzGerald
GA: Increased thromboxane biosynthesis in normal preg-
nancy is mainly derived from platelets. Am J Obstet Gynecol
1987;157:325–330
35. Falk E: Unstable angina with fatal outcome: Dynamic cor-
orary thrombosis leading to infarction and/or sudden death.
LM, Willerson JT: Mediation of reocclusion by thrombox-
ane A2 and serotonin after thrombosis with tissue-type
plasminogen activator in a canine model of coronary throm-
coronary occlusion in acute myocardial infarction: Value of
combined thrombolytic therapy and vasodilator therapy. N

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