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 A$_2$ biosynthesis during coronary thrombolysis with intravenous t-PA in patients with acute myocardial infarction. Urinary 2,3-dinor-thromboxane B$_2$, a metabolite of thromboxane A$_2$, 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 A$_2$ rather than washout of preformed metabolites during reperfusion. Prostacyclin (PGI$_2$) biosynthesis, determined by excretion of 2,3-dinor-6-keto-PGF$_{1\alpha}$, 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 PGI$_2$ 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. In particular, platelets delay reperfusion and induce reocclusion. Platelet activation is also increased in patients with acute myocardial infarction treated with intravenous streptokinase, and antiplatelet therapy enhances the response to streptokinase, accelerating reperfusion and further reducing mortality. However, streptokinase directly activates human platelets. In contrast, t-PA inhibits platelet function in vitro. Although t-PA has been reported to increase platelet aggregation ex vivo, this increase may reflect an artifact due to plasminogen activation enhanced by fibrin formed during sampling and a proaggregatory response to plasmin. 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) A$_2$ biosynthesis as an index of platelet activation during coronary thrombolysis with t-PA in humans. TxA$_2$, 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. We also determined biosynthesis of prostacyclin (PGI$_2$), a major cyclo-oxygenase product of arachidonic acid in endothelium and a potent platelet inhibitor. Formation of these eicosanoids is increased in conditions associated with platelet activation, including unstable angina, acute myocardial infarction, and severe peripheral vascular disease, 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 PG12 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 PG12 formation by isolated vascular tissue.17 However, we have demonstrated that nitrates do not alter PG12 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-
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.i. 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 angiography 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±14×10^9/μl to 248±12×10^9/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-

![Graph showing urinary excretion of 2,3-dinor-TxB₂](http://circ.ahajournals.org/)

**FIGURE 1.** Plots of urinary excretion of 2,3-dinor-TXB₂ (TX-M, pg/mg creatinine) in patients with acute myocardial infarction who had received intravenous t-PA alone (n=12) or combined with oral aspirin (ASA), 325 mg (n=6).
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.³¹ 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.³²

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,³³ 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 TxA₂.¹⁵,³²,³⁴ 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 unstable angina and a previously reported group of patients with acute myocardial infarction who did not receive thrombolytic therapy.¹⁴ TxA₂ biosynthesis in patients receiving thrombolytic therapy greatly exceeded that seen in patients with unstable angina, a condition characterized by coronary thrombosis³⁵ 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.⁵,¹⁴ 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 intracoronal streptokinase in humans.⁶ 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.⁴ 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. These findings suggest that TxA2 can impair the clinical response to thrombolytic therapy. TxA2 is a potent platelet agonist and may be a mediator of platelet activation during coronary thrombolyis, as demonstrated in animal models. TxA2 may also induce vasospasm during coronary thrombolyis and may be a determinant of infarct size in the setting of reperfusion.

This study also demonstrates that patients who reperfused with t-PA fail to show the normal infarct-related rise in PGI2 biosynthesis, as determined by excretion of its enzymatic metabolite, 2,3-dinor-6-keto-PGF1α. In patients with acute myocardial infarction who are not receiving a thrombolytic agent, PGI2 formation rose over the 48 hours after admission and gradually declined toward normal by day 5. 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 PGI2 biosynthesis derives from the heart. 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 PGI2 in myocardial tissue. Furthermore, injured myocytes release arachidonic acid, which may be metabolized by other cells to form prostaglandins including PGI2.

Although the number of patients was small, a similar increase in PGI2 was seen in patients who failed to reperfuse after t-PA administration. In contrast, the increase in PGI2 biosynthesis was blunted in patients who reperfused, and it did not correlate with plasma CPK. The normal prolonged increase in PGI2 biosynthesis also fails to occur following reperfusion with streptokinase. The mechanism of the reduction in PGI2 biosynthesis after coronary reperfusion is uncertain. Reperfusion may reduce infarct size and, as a consequence, PGI2 biosynthesis. However, because CPK release correlates with infarct size, even in the setting of reperfusion, there would continue to be a relation between plasma CPK and PGI2 formation. In contrast, no correlation was found between these variables after reperfusion with t-PA. An alternative explanation is that the decrease in PGI2 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. Recent studies in vitro suggest that this may be mediated by oxygen free radicals generated during reperfusion and reoxygenation of the ischemic endothelium. Thus, the failure to increase PGI2 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 thrombolyis 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 or by the continued formation of thrombin. This process may explain the increased TxA2 formation seen even in patients who fail to reperfuse. Further activation may occur with exposure of platelets to the injured vascular bed after reperfusion. 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 U/ml), which are not achieved in vivo. Thus, plasma α2-antiplasmin, which forms 1:1 stoichiometric complexes with plasmin, decreases by only 25% after t-PA administration in humans. Therefore, the systemic concentration of plasmin in vivo is unlikely to be sufficient to induce platelet activation.

In conclusion, platelet activation is increased during coronary thrombolyis 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 thrombolyis with t-PA is TxA2 because biosynthesis of this eicosanoid is increased. In addition, these data demonstrate a reduction in PGI2 biosynthesis in patients who reperfuse. This may be a noninvasive marker of reperfusion injury in humans and may amplify further platelet activation during coronary thrombolyis. These data support the further evaluation of antiplatelet therapy and TxA2 inhibition during coronary thrombolyis with t-PA.

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