Marked platelet activation in vivo after intravenous streptokinase in patients with acute myocardial infarction

DESMOND J. FITZGERALD, M.B., FRANCESCA CATELLA, M.D., LOUIS ROY, M.D., AND GARRET A. FITZGERALD, M.D.

ABSTRACT We assessed thromboxane biosynthesis as an index of platelet activation in 6 patients with acute myocardial infarction receiving intravenous streptokinase. Urinary 2,3-dinor-thromboxane B2 and plasma 11-dehydro-thromboxane B2, major enzymatic metabolites of thromboxane A2, were markedly increased after intravenous streptokinase (11,063 ± 2758 pg/mg creatinine and 33 ± 10 pg/ml, respectively) compared with values in patients not receiving thrombolytic therapy (502 ± 89 pg/mg creatinine and 3 ± 0.7 pg/ml). Prostacyclin biosynthesis also increased markedly after streptokinase coincident with the increase in thromboxane A2 formation. Administration of aspirin between the time of onset of coronary thrombosis and reperfusion both in man and in a canine preparation demonstrated that this reflected thromboxane biosynthesis de novo and not metabolism of preformed inactive thromboxane B2 washed out from the coronary circulation. Since the platelet is the major source of thromboxane A2, these findings suggest that there is marked platelet activation after coronary thrombosis with streptokinase. Studies in vitro demonstrated that streptokinase enhanced platelet activation in a dose-dependent manner, resulting in the secondary release of thromboxane A2. The increase in platelet activation and thromboxane A2 biosynthesis may limit the therapeutic effect of intravenous streptokinase in acute myocardial infarction.


THE CLINICAL RESPONSE to intravenous streptokinase in patients with acute myocardial infarction may be limited by failure to reperfuse the occluded coronary artery despite evidence of a systemic lytic effect.1–4 Even in patients who reperfuse initially the clinical outcome is often complicated by a residual high-grade stenosis5, 6 and by early reocclusion.7, 8 Evidence from studies in experimental animal preparations suggest that this may in part be due to platelet activation since interventions that inhibit platelet function enhance the thrombolytic response to streptokinase9 and prevent reocclusion.10 Whether platelet activation also plays a role in modulating the response to streptokinase in patients with acute myocardial infarction is unknown.

We have previously demonstrated increased biosynthesis of thromboxane A2 and prostacyclin, the major cyclooxygenase products of arachidonic acid, in platelets and vascular endothelium, respectively, in a number of conditions in which platelets are known to play a role,11–14 suggesting that measurement of these eicosanoids reflects platelet activation in vivo. In this study, we determined thromboxane A2 and prostacyclin biosynthesis as indexes of platelet activation in patients with acute myocardial infarction receiving streptokinase.

Methods

Patients not treated with aspirin. The study group consisted of patients admitted with chest pain of cardiac origin who had electrocardiographic evidence of an acute myocardial infarction. Patients were excluded if they had taken aspirin or any other cyclooxygenase inhibitors in the 10 days before admission. Streptokinase (Kabikinase, Kabivitrum, Alameda, CA) was administered intravenously in a dose of 750,000 to 1,500,000 units over 30 to 60 min. All patients received anticoagulant therapy with heparin after completion of the streptokinase infusion. In addition, all of the subjects received sublingual and intravenous nitroglycerin before and after the administration of streptokinase. Urine was collected over each 6 hr period for up to 60 hr for determination of 2,3-dinor-6-keto-prostaglandin F1α.
and 2,3-dinor-thromboxane B₂, major enzymatic metabolites of prostacyclin and thromboxane A₂, respectively. In addition, plasma was obtained 2 to 4 hr after starting the streptokinase infusion for measurement of 11-dehydro-thromboxane B₂, the most abundant enzymatic metabolite of thromboxane A₂ in plasma. Plasma creatinine kinase was determined repeatedly in the first 24 hr as an index of reperfusion. Patients receiving streptokinase were compared with patients with acute myocardial infarction studied concurrently who did not receive thrombolytic therapy. The clinical characteristics and part of the metabolite data (peak levels only) for this latter group have been published previously. The control patients differed in that they presented later than 4 hr after the onset of chest pain but were similar with respect to the distribution of infarcts. The protocol was approved by the Committee for the Protection of Human Subjects at Vanderbilt University and all subjects gave informed written consent.

**Aspirin studies.** To address the possibility that the increase in thromboxane A₂ metabolite excretion reflected metabolism of preformed inactive thromboxane B₂ washed out from the coronary circulation and not an increase in thromboxane A₂ biosynthesis, we examined the effect of aspirin administered immediately before thrombolytic therapy on thromboxane metabolite formation in two patients with acute myocardial infarction and in a canine preparation of coronary thrombosis. A No. 8F sheath was implanted in the right carotid artery of mongrel dogs anesthetized with 30 mg/kg pentobarbital and ventilated with a Harvard respirator. A 4 to 5 mm copper coil was implanted in the circumflex coronary artery by advancing it over a guide with use of a No. 5F catheter and the catheter and guide were removed, leaving the coil in place. This resulted in the formation of an occlusive thrombus in 10 to 15 min. Two hours after formation of the thrombus 20 mg/kg aspirin (n = 3) or vehicle (n = 3) was administered intravenously over 10 min. This dose of aspirin resulted in greater than 92% inhibition of serum thromboxane B₂. After a further 2 hr period of reperfusion was induced with intravenous or 20,000 units/kg intracoronary streptokinase (Kabikinase) infused over 30 min and 200 units/kg heparin administered intravenously, and was confirmed angiographically on completion of the study. Urine was collected before implantation of the coil and hourly after induction of coronary thrombosis for determination of 2,3-dinor-thromboxane B₂. The protocol for canine studies was reviewed and approved by the Animal Care Committee at Vanderbilt University.

**Platelet studies.** The effect of streptokinase on aggregation of human platelets was determined at 37°C by light transmission18 with a four-channel aggregometer (Pap-4, Bio-data Corporation, Harboro, PA). Peripheral venous blood from normal volunteers was drawn into a syringe containing 3.8% sodium citrate to a final dilution of 9:1. Platelet-rich plasma was prepared by centrifugation of the citrated blood for 15 min at 100 g. After separation of the platelet-rich plasma, platelet-poor plasma was prepared by centrifugation of the remaining blood at 800 g for 10 min and the platelet count of the platelet-rich plasma was adjusted to 300,000/ml with platelet-poor plasma. Streptokinase from two sources (Kabivitrum and Sigma, St. Louis, MO) was studied and the results with both preparations were found to be indetical. Streptokinase was dissolved in 0.9% sodium chloride immediately before being used and was added to the cuvette in volumes of 10% or less of the platelet preparation. Aggregations were performed in 200 to 500 μl of the platelet preparations with aggregating agents (ADP 1 to 2 μM, epinephrine 1 to 2 μM) being added in volumes of 5% or less. All concentrations refer to the final concentration in platelet-rich plasma.

**Biochemical studies.** Biosynthesis of thromboxane A₂ and prostacyclin was determined by measurement of their enzymatic metabolites, which minimizes the artifact induced by sampling. Urine was analyzed for 2,3-dinor-thromboxane B₂ and 2,3-dinor-6-keto-prostaglandin F₃₀ and plasma was analyzed for 11-dehydro-thromboxane B₂ by highly specific and sensitive assays with quantitation by negative ion, capillary column-gas chromatography–mass spectrometry as previously described. Thromboxane B₂ was determined by radioimmunoassay in serum obtained from blood incubated in glass at 37°C for 45 min and in platelet-rich plasma frozen rapidly at −70°C 6 min after the addition of the platelet agonist. For measurement of fibrinogen in platelet-rich plasma, 900 Kallikrein units/ml aprotinin (Sigma) was added and the samples were rapidly cooled before separation of the platelets by centrifugation. The plasma was stored at −20°C and analyzed for fibrinogen and its clottable degradation products within 12 hr, as previously described. Plasminogen concentration in citrated plasma was determined after activation with excess streptokinase in the presence of the chromogenic substrate H-d-Valyl-L-cyclohexyl tyrosyl-L-lysine paranitronanilide (Chromostrase, General Diagnostics, Morris Plains, NJ) and subsequent measurement of absorbance at 405 nm.

**Statistical analysis.** Urinary metabolite excretion and plasma 11-dehydro-thromboxane B₂ in patients receiving streptokinase were compared with those in patients with acute myocardial infarction not receiving thrombolytic therapy who were studied concurrently. Comparisons between groups were by Wilcoxon rank-sum tests for paired and unpaired data where appropriate. These are nonparametric tests and therefore make no assumption as to the distribution of the data.

**Results**

Six male patients (age 48 ± 3.6 years) received streptokinase without pretreatment with aspirin (table 1) and were compared with 14 male patients (age 61 ± 2.6 years) with acute myocardial infarction who did not receive thrombolytic therapy. Additional management was similar in the two groups during the period of study, except that only two of the control patients received heparin. None of these patients underwent cardiac catheterization during the period of sample collection and the other types of drug treatment were similar in the two groups (table 2). None of the patients developed cardiogenic shock or pulmonary edema. Of the six patients receiving streptokinase, four showed evidence of reperfusion based on symptoms, electrocardiographic changes, and peak plasma levels of creatine kinase occurring 12 hr or less after the infusion of streptokinase. Angiography at 2 to 3 days demonstrated a patent vessel in three of these patients. In the fourth patient, despite a rapid resolution of symptoms and an early peak in plasma creatine kinase, the left anterior descending coronary artery was occluded at catheterization 5 days later. This patient had a further episode of chest pain before catheterization, suggesting that reocclusion may have occurred after initial reperfusion. In one patient there was no evidence of reperfusion based on clinical, electrocardiographic, or biochemical data. Angiography 3 days later demonstrated an

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TABLE 1
Clinical and angiographic findings in patients with acute myocardial infarction receiving streptokinase (SK)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Infarct site</th>
<th>Onset of symptoms to SK infusion (min)</th>
<th>Peak CK (unit/ml)</th>
<th>Time to Peak CK (hr)</th>
<th>Angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>No aspirin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>Anterior</td>
<td>135</td>
<td>2180</td>
<td>7</td>
<td>LAD occluded</td>
</tr>
<tr>
<td>52</td>
<td>Anterolateral</td>
<td>180</td>
<td>1710</td>
<td>23</td>
<td>LAD occluded</td>
</tr>
<tr>
<td>47</td>
<td>Anterolateral</td>
<td>120</td>
<td>3100</td>
<td>7</td>
<td>LAD 40% stenosis</td>
</tr>
<tr>
<td>58</td>
<td>Anterior</td>
<td>180</td>
<td>1344</td>
<td>13</td>
<td>No cath</td>
</tr>
<tr>
<td>53</td>
<td>Anterior</td>
<td>145</td>
<td>1938</td>
<td>10</td>
<td>LAD 70% stenosis</td>
</tr>
<tr>
<td>48</td>
<td>Lateral</td>
<td>120</td>
<td>1200</td>
<td>8</td>
<td>OM 90% stenosis</td>
</tr>
<tr>
<td>Aspirin pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Inferior</td>
<td>120</td>
<td>4980</td>
<td>8</td>
<td>RCA 40% stenosis</td>
</tr>
<tr>
<td>65</td>
<td>Anterior</td>
<td>145</td>
<td>5760</td>
<td>8</td>
<td>LAD 80% stenosis</td>
</tr>
</tbody>
</table>

CK = creatine kinase; LAD = left anterior descending coronary artery; OM = obtuse marginal coronary artery; RCA = right coronary artery.

occluded left anterior descending coronary artery. In the sixth patient it was uncertain whether reperfusion had occurred, since while symptoms had resolved abruptly and ventricular arrhythmias developed during the streptokinase infusion, peak plasma creatine kinase occurred at between 12 and 18 hr. Angiography was not performed in this patient.

Urinary 2,3-dinor-thromboxane B₂, a major enzymatic metabolite of thromboxane, was markedly increased after the infusion of streptokinase, the median (and range) in the first urine collection being 9356 (3447 to 19,445) pg/mg creatinine compared with 408 (128 to 1086) pg/mg creatinine in patients with an acute myocardial infarction not receiving thrombolytic therapy (figure 1; p < .001). The highest level was seen in the first urine collection and fell rapidly thereafter but remained above normal for at least 24 hr. The upper 95% confidence limit normal volunteers is 350 pg/mg creatinine. Consistent with the urinary data, plasma 11-dehydro-thromboxane B₂ was 33 ± 10 pg/ml (n = 4) in patients receiving streptokinase compared with 3.0 ± 0.7 pg/ml (n = 4) in patients with acute myocardial infarction not receiving thrombolytic therapy (figure 1; p < .05). Similarly, urinary 2,3-dinor-6-keto-prostaglandin F₃α was increased after infusion of streptokinase and fell rapidly over the next 24 hr in all but one subject (figure 2). This subject had failed to reperfuse and showed a marked and late increase in the urinary excretion of this metabolite.

In two additional patients with acute myocardial infarction, plasma and urinary thromboxane metabolites were also determined immediately before the administration of streptokinase. In both patients there was a marked increase in urinary 2,3-dinor-throm-
boxane B₂ and in plasma 11-dehydro-thromboxane B₂ after administration of streptokinase (figure 3).

To address the possibility that the increase in thromboxane A₂ metabolites after thrombolytic therapy reflected washout and subsequent metabolism of inactive thromboxane B₂ from the thrombus and not an increase in thromboxane A₂ biosynthesis, we examined the effect of aspirin on thromboxane A₂ formation in a canine preparation of coronary thrombosis and reperfusion. Induction of thrombosis was associated with an increase in urinary 2,3-dinor-thromboxane B₂, which subsequently declined. After reperfusion with intravenous streptokinase (n = 3) there was a further increase in urinary 2,3-dinor-thromboxane B₂. When aspirin was administered 2 hr after the induction of coronary thrombosis but before the infusion of streptokinase (n = 3), no increase in 2,3-dinor-thromboxane B₂ occurred after reperfusion (figure 4).

In addition, we studied the effect of aspirin in two patients with acute myocardial infarction treated with intravenous streptokinase. One patient had taken 325 mg aspirin after the onset of chest pain but before reaching the hospital and receiving streptokinase 1,500,000 units intravenously 2 hr later. Reperfusion was suggested by the abrupt cessation of chest pain and the appearance of an accelerated idioventricular rhythm 1 hr after the onset of the infusion and was confirmed by an early peak in plasma creatinine kinase (figure 5) and by the coronary angiogram obtained 48 hr after admission. The second patient, who presented with an acute anterior myocardial infarction, received 325 mg aspirin by mouth immediately before the infusion of streptokinase. Reperfusion was confirmed 2 hr later by coronary angiography. Urinary 2,3-dinor-6-keto-prostaglandin F₁α (pg/mg creatinine)
Aggregation induced in vitro by 1 to 2 μM ADP. The proaggregatory and fibrinogenolytic effects of streptokinase are blocked by aprotinin and ε-aminocaproic acid (ε-ACA).

To examine the effect of streptokinase in the absence of plasminogen, citrated platelet-rich plasma was passed over a lysine-Sepharose (Sigma) column, as previously described for plasma, before platelet aggregation studies (n = 3). This resulted in complete removal of plasminogen, whereas the platelet count decreased by only 30% and fibrinogen concentration was unaltered. Removal of plasminogen from plasma prevented the proaggregatory effect of streptokinase, whereas platelet aggregation to ADP was unaltered (figure 7). Consistent with a requirement of plasminogen, the proaggregatory effect of streptokinase was again demonstrable after readdition of exogenous human plasminogen in a concentration of 91 mg/dl (figure 7).

Aggregation induced by streptokinase was associated with a marked increase in thromboxane A2 formation as measured by thromboxane B2, its inactive hydrolysis product (figure 8). However, this did not mediate the proaggregatory effect since neither aspirin, which blocked the formation of thromboxane B2, nor 3-carboxy-dibenzo (b,f) thiepin-5,5-dioxide (L636, 499, a generous gift of Dr. A. Ford-Hutchinson, Merck-Frost, Canada), a specific thromboxane A2/prostaglandin endoperoxide receptor antagonist,26 blocked this effect (figure 8).

**Discussion**

This study demonstrates a marked increase in thromboxane A2 metabolites in plasma and urine in patients with acute myocardial infarction who had received intravenous streptokinase compared with patients not receiving thrombolytic therapy. Although the control patients differed in that they presented later and their peak urinary metabolite excretion may have occurred.
before entry into the study, this is unlikely to explain the differences between groups. Thus, thromboxane metabolite excretion after streptokinase administration grossly exceeds the levels seen during the development of an acute myocardial infarction or in patients presenting within 4 hr of the onset of pain who were not given thrombolytic therapy. Furthermore, in two patients urine and plasma were obtained before and after administration of streptokinase. In both cases plasma 11-dehydro-thromboxane B₂ and urinary 2,3-dinor-thromboxane B₂ on admission were similar to the levels in control patients and increased markedly after administration of streptokinase (figure 3). Control patients also differed in that the majority did not receive heparin. However, in two patients with acute myocardial infarction who received heparin alone, peak urinary 2,3-dinor-thromboxane B₂ levels were 334 and 1086 pg/mg creatinine, well below the level seen in patients who also received streptokinase. In addition, heparin does not increase thromboxane A₂ biosynthesis in patients with unstable angina.* 

That the increase in urinary 2,3-dinor-thromboxane B₂ reflected an increase in thromboxane A₂ biosyn-

*Fitzgerald DJ, FitzGerald GA: Unpublished observation.
thesis and not washout and subsequent metabolism of thromboxane B₂, the inactive hydrolysis product of thromboxane A₂, from the coronary circulation is supported by a number of findings. First, the increase in urinary 2,3-dinor-thromboxane B₂ appeared unrelated to reperfusion. Thus, the increase in urinary metabolite excretion persisted for up to 24 hr after the administration of streptokinase and the highest level was seen in one patient in whom reperfusion failed to occur. Second, in a canine preparation of coronary thrombosis associated with a marked increase in thromboxane A₂ generation, thrombolysis and reperfusion failed to result in the appearance of thromboxane B₂ metabolites in urine when biosynthesis of thromboxane A₂ was inhibited by aspirin. Similarly, no increase in urinary 2,3-dinor-thromboxane B₂ was seen in two patients with acute myocardial infarction treated with aspirin between the onset of symptoms and subsequent administration of streptokinase. Thus, the increase in thromboxane A₂ metabolites after administration of streptokinase in patients with acute myocardial infarction reflects thromboxane A₂ biosynthesis de novo and not metabolism of inactive thromboxane B₂ washed out from the coronary circulation.

Since the platelet is the major source of thromboxane A₂ under physiologic conditions in man, these findings suggest that thrombolytic therapy with streptokinase results in marked platelet activation. Consistent with this hypothesis, aspirin in a single dose of 325 mg, which results in greater than 95% inhibition of platelet cyclooxygenase, 28 prevented the increase in thromboxane biosynthesis after streptokinase in two patients with acute myocardial infarction. In contrast, prostacyclin biosynthesis, determined by measurement of its major enzymatic metabolite 2,3-dinor-6-keto-prostaglandin F₁₀, was increased to a degree similar to that seen in patients not receiving aspirin. This is not unexpected, since a single 325 mg dose of aspirin would result in incomplete and reversible inhibition of tissue cyclooxygenase, allowing prostacyclin biosynthesis to increase. 28 Thus, the increase in thromboxane A₂ biosynthesis in patients with acute myocardial infarction receiving streptokinase is largely, and perhaps completely, derived from platelets.

Urinary 2,3-dinor-6-keto-prostaglandin F₁₀ also increased early after streptokinase in all but one patient who failed to reperfuse and in whom there was a late and more marked increase in excretion of this metabolite. This is consistent with our previous data demonstrating a late and marked rise in prostacyclin biosynthesis in patients with acute myocardial infarction not receiving thrombolytic therapy. 11 This delayed increase in prostacyclin formation may reflect myocardial necrosis since it was temporarily related to the rise in plasma creatine kinase. 11 In contrast, prostacyclin biosynthesis increased early in patients in whom reperfusion was successful, before the increase in plasma creatine kinase and at a time when thromboxane A₂ biosynthesis was maximal. We have previously demonstrated increased prostacyclin biosynthesis coincident with increased thromboxane A₂ formation in a number of conditions associated with platelet activation, including unstable angina, 11 severe atherosclerosis, 12, 13 and systemic sclerosis, 14 consistent with the hypothesis that this reflects a compensatory response to platelet activation in vivo. Thus, the increase in prostacyclin biosynthesis in our study group is further evidence of platelet activation after administration of streptokinase in the presence of acute myocardial infarction.

Increased thromboxane A₂ biosynthesis after the administration of streptokinase could reflect either a direct effect of the drug on platelets or platelet activation secondary to thrombolysis and/or coronary reperfusion. In this study, we demonstrated that streptokinase enhances platelet activation to a range of platelet agonists in vitro. This occurred at concentrations that were at the threshold for a fibrinogenolytic effect and the phenomenon was prevented by the serine protease inhibitors aprotinin and ε-aminocaproic acid. These findings suggest that the proaggregatory effect of streptokinase is closely related to its fibrinogenolytic activity. Platelet activation may therefore occur at the doses used to treat acute myocardial infarction, which are associated with a profound systemic fibrinogenolytic effect. 29 This is further supported by an increase in platelet activation ex vivo in patients receiving thrombolytic therapy 30 and in a rabbit preparation of pulmonary embolism after administration of streptokinase. 31

The mechanism of this proaggregatory effect is unknown. Despite an increase in thromboxane A₂ formation coincident with streptokinase-induced platelet activation, inhibition of its formation with aspirin or antagonism of its effect with the thromboxane A₂/prostaglandin endoperoxide receptor antagonist L636,499 failed to inhibit platelet aggregation. Thus, release of thromboxane A₂ appears to be a secondary response. Recently, Shafer et al. 32 demonstrated that plasmin, which is formed by the action of the streptokinase-plasmin complex on plasminogen, could activate platelets at high concentrations. 32 Consistent with a role for plasmin in mediating the platelet response to streptokinase, the proaggregatory effect of this throm-
bolytic agent was abolished in plasminogen-depleted platelet-rich plasma, but was reinstituted on addition of exogenous plasminogen. Increased platelet activation after streptokinase administration may also reflect mechanisms independent of its direct effect on platelets, including platelet activation by exposed subendothelial collagen at the site of the coronary lesion or by procoagulant systems on the clot surface,33 or in the ischemic coronary bed after reperfusion.34

The pathophysiologic importance of increased platelet activation after administration of streptokinase in patients with acute myocardial infarction is uncertain. Animal studies suggest that platelet activation does modulate the response to streptokinase and other thrombolytic agents in vivo, both in limiting reperfusion and in mediating early reocclusion.9, 10, 34 In addition, platelet products released after activation may have adverse physiologic effects. The increase in biosynthesis of thromboxane A2, a potent platelet agonist and vasoconstrictor,35 in our study group far exceeds the level in patients with unstable angina11 in whom studies with aspirin imply its functional importance.36, 37 Thus, thromboxane A2 released after platelet activation by streptokinase may recruit other platelets. Furthermore, there is evidence to suggest that vasoactive platelet products released after administration of streptokinase may limit effective reperfusion31, 38, 39 and that thromboxane A2 generated during platelet activation can induce local vasoconstriction.40 Whether these factors limit the response to streptokinase in acute myocardial infarction in humans is not known. The effect of aspirin on the response to this therapy is presently being studied in a multicenter trial.41

In conclusion, administration of streptokinase in patients with acute myocardial infarction induces a massive increase in thromboxane A2 biosynthesis that largely results from platelet activation. Such a phenomenon may limit the effectiveness of intravenous streptokinase in inducing clot lysis and reperfusion in humans.

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Vol. 77, No. 1, January 1988
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