Platelet Function During and After Thrombolytic Therapy for Acute Myocardial Infarction With Reteplase, Alteplase, or Streptokinase

Martin Moser, MD; Thomas Nordt, MD; Karlheinz Peter, MD; Johannes Ruef, MD; Benedikt Kohler, MD; Marc Schmittner, BA; Richard Smalling, MD, PhD; Wolfgang Küberl, MD; Christoph Bode, MD

**Background**—Changes in platelet aggregation (PA) and platelet surface receptor expression induced by thrombolytic therapy for acute myocardial infarction may influence the rate of initial reperfusion and early reocclusion.

**Methods and Results**—In the RAPID-1 (Reteplase Angiographic Phase II International Dose-finding study), RAPID-2 (Reteplase vs Alteplase Patency Investigation During myocardial infarction), INJECT (International Joint Efficacy Comparison of Thrombolytics), and GUSTO-3 (Global Use of Strategies To Open occluded coronary arteries) trials, 126 patients were enrolled in a single center. Patients were treated with either conventional alteplase (100 mg/180 min; n = 15), accelerated alteplase (100 mg/90 min; n = 21), reteplase 10+10-U double bolus (n = 50), reteplase 10+5-U double bolus (n = 15), reteplase 15-U single bolus (n = 15), or streptokinase (1.5 MU/60 min; n = 10). PA (after stimulation with ADP), P-selectin expression and fibrinogen binding to glycoprotein (GP) IIb/IIIa (determined by flow cytometry with and without stimulation with ADP), and levels of soluble P-selectin, prothrombin fragments F1 and F2, thrombin-antithrombin complexes (TAT), and antithrombin III (ATIII) were determined. PA decreased significantly at 1 and 2 hours in patients treated by 10+10-U reteplase or by streptokinase. Fibrinogen binding to platelet GP IIb/IIIa followed a similar pattern. Significant thrombin generation and significantly elevated thrombin levels during thrombolysis were reflected by increased F1 and F2 fragments and TAT levels in all treatment groups. ATIII levels decreased significantly during thrombolytic therapy.

**Conclusions**—A decrease in PA in patients treated by reteplase or streptokinase compared with alteplase could be observed in the early phase. Double bolus (10+10 U) reteplase and streptokinase resulted in lower PA at 1 and 2 hours than therapy with accelerated alteplase. Total fibrinogen and fibrinogen binding to GP IIb/IIIa tended to be lower during the first 2 hours after reteplase than after accelerated alteplase. *(Circulation. 1999;100:1858-1864.)*

**Key Words:** thrombolysis ■ platelet aggregation inhibitors ■ plasminogen activators ■ myocardial infarction ■ reperfusion

Various thrombolytic agents have been developed in the past decade to achieve higher early patency rates of the infarct-related coronary artery and to improve survival. Recombinant tissue plasminogen activator (alteplase) has proved more effective with regard to establishing TIMI (Thrombolysis In Myocardial Infarction) grade III flow than streptokinase. Reteplase, a nonglycosylated deletion mutant lacking the finger, growth factor, and kringle-1 domains of alteplase, has a prolonged half-life of 18 minutes, which allows for double-bolus application. Reteplase has been compared with alteplase in 2 patency studies, RAPID-1 (Reteplase Angiographic Phase II International Dose-finding study) and RAPID-2 (Reteplase vs Alteplase Patency Investigation During myocardial infarction), and with streptokinase and alteplase in the mortality trials INJECT (International Joint Efficacy Comparison of Thrombolytics trial) and GUSTO-3 (Global Use of Strategies to Open occluded coronary arteries).

Platelets play a key role in the pathogenesis of acute coronary syndromes. After an atherosclerotic plaque is disrupted, its thrombogenic lipid core becomes exposed to the plasma, and adjacent platelets begin to form an occlusive thrombus. This process involves platelet activation that leads to conformational changes of the platelet receptor integrin glycoprotein (GP) IIb/IIIa. Activated GP IIb/IIIa binds fibrinogen, which forms interplatelet bridges and thus causes platelet aggregation. During activation of platelets, degranulation of α-granules occurs. In the process, the...
α-granule protein P-selectin is exposed to the platelet surface. P-selectin plays an important role in the interaction between platelets and both endothelium and leukocytes.

In the present investigation, platelet aggregation, fibrinogen binding to GP IIb/IIIa, and activation of platelets reflected by surface expression and plasma levels of P-selectin in patients with acute myocardial infarction were studied before, during, and after treatment with different thrombolytic agents and regimens. Patients recruited in the present study represent a single-center subgroup of patients enrolled in the RAPID-1, RAPID-2, INJECT, and GUSTO-3 trials. The aim of the study was to investigate platelet function before, during, and after treatment with different thrombolytic regimens.

Methods

Patients

Between August 1991 and January 1997, 131 patients with acute myocardial infarction admitted to the intensive care unit of the Universitätsklinik Heidelberg were enrolled in the RAPID-1, RAPID-2, INJECT, or GUSTO-3 trial. All patients were at least 18 years old and underwent thrombolytic treatment <6 hours (12 hours in RAPID-2 and INJECT) after the onset of typical chest pain. ECG criteria for enrollment consisted of either ST-segment elevation ≥0.1 mV in 2 of the inferior or lateral leads, ST-segment elevation ≥0.2 mV in ≥2 contiguous precordial leads, or the presence of left bundle-branch block. Patients were not enrolled if they had a history of PTCA within the preceding 2 weeks or a history of any condition conventionally precluding thrombolytic therapy. Women of childbearing potential were excluded as well. The study protocols were approved by the institute’s ethics committee, and written informed consent was obtained from all patients before thrombolytic therapy was begun.

Study Designs and Treatment

All patients received thrombolytic therapy according to the respective study protocol. Adjunctive therapy consisted of aspirin (250 mg given intravenously immediately before initiation of thrombolytic therapy and 100 mg orally once per day starting the following day) and heparin (5000 U given intravenously before thrombolytic therapy and continued at a dose of ~1000 U/h for ≥24 hours). The dose of heparin was titrated to maintain the activated partial thromboplastin time (aPTT) between 60 and 80 seconds (1.7 to 2.3 times the control value). Other medications, including β-blocking agents, were administered at the discretion of the treating physician.

RAPID-1

This study was a multicenter, open-label, parallel-group study in which patients were randomized to receive a 15-U single bolus of reteplase, a 10-U bolus of reteplase followed by a 5-U bolus 30 minutes later (15 U total dose), a 10-U bolus of reteplase followed by a 10-U bolus 30 minutes later (20 U total dose), or alteplase 60 mg over the first hour, with 6 to 10 mg being administered as an initial bolus followed by 20 mg/h for an additional 2 hours (maximum total dose 100 mg). After initiation of thrombolytic treatment, the patient was taken to the catheterization laboratory, and coronary angiography was performed at 30 and 60 minutes (if possible) and at 90 minutes (mandatory).10

RAPID-2

This study was a multicenter, open-label, parallel-group study in which eligible patients were randomized to receive either a double bolus of reteplase (a 10-U bolus given at the start of therapy followed by a 10-U bolus 30 minutes later) or an accelerated alteplase regimen: 15-mg bolus, 0.75 mg/kg over 30 minutes (maximum 50 mg), and 0.5 mg/kg over 60 minutes (maximum 35 mg). Coronary angiography was performed at 30, 60 (if possible), and 90 minutes (mandatory) after the initiation of thrombolytic therapy.4

INJECT

This multicenter trial was double-blinded with a double-dummy procedure: either 2 boluses of reteplase (10 U each) given 30 minutes apart plus a 1-hour placebo infusion or placebo boluses plus a 1-hour infusion containing 1.5 MU of streptokinase were administered.

GUSTO-3

This study was a multicenter, open-label study in which patients were randomized to receive either a double-bolus reteplase regimen or an accelerated alteplase regimen as described for RAPID-2.

Platelet Aggregation

For the determination of platelet aggregation and other hemostatic variables, peripheral blood samples were collected from an antecubital vein into sodium citrate (final concentration 0.011 mol/L) immediately before the initiation of thrombolytic therapy (0 h) and 1, 2, and 12 hours thereafter. Blood samples for the measurement of ex vivo platelet aggregation were centrifuged at 160g for 10 minutes. The supernatant, platelet-rich plasma (PRP), was removed, and the remaining blood underwent a second centrifugation step at 2500g for 10 minutes to obtain platelet-poor plasma (PPP). PRP was diluted with autologous PPP to adjust platelet count in PRP to 250/mL. All samples were centrifuged and stored at room temperature to avoid premature platelet activation.

Platelet aggregation was determined by light transmission in a 4-channel aggregometer (PAP-4, Biodata Corporation). The aggregometer was adjusted before each test with light transmission of PRP corresponding to 0% and that of PPP corresponding to 100%. Platelets in 200 µL of PRP at 37°C were stimulated by the addition of ADP (final concentrations of 2, 10, and 20 µmol/L) or collagen (final concentration of 1.5 µg/mL) and stirred at 900 rpm. Each aggregation curve was registered for a minimum of 5 minutes. Aggregation was quantified by maximal initial slope (change of light transmission over time) and maximal total increase in light transmission. Platelet aggregation was assessed between 50 and 70 minutes after collection of the blood sample. Investigations performed in our laboratory have shown that platelet aggregation is stable between 30 and 120 minutes after collection of the blood sample (data not shown).

Prothrombin Fragments F1 and F2, Thrombin-Antithrombin Complexes, and Antithrombin III

PPP samples were stored at −70°C until assay. Plasma concentrations of prothrombin fragments F1 and F2 were determined by an enzyme immunoassay according to the sandwich principle (Enzygnost F1+2 micro, Behring). The reference range was 0.4 to 1.1 nmol/L, with coefficients of variation of 5% to 7.5% (intra-assay) and 6% to 13% (interassay), respectively.

Plasma concentrations of thrombin-antithrombin complexes (TAT) were measured by a sandwich enzyme immunoassay (Enzygnost TAT, Behring). The reference range was 1.0 to 4.1 µg/L, with coefficients of variation of 2% to 6% (intra-assay) and 2.4% to 12.0% (interassay), respectively. For the determination of antithrombin III (ATIII) activity, plasma samples were incubated with an excess amount of thrombin in the presence of heparin. Residual thrombin activity was assayed spectrophotometrically at 405 nm in a kinetic test (Berichrom Antithrombin III, Bering). The reference range was 75% to 125%, with coefficients of variation of 2.8% to 4.4% (intra-assay) and 4.8% to 6.2% (interassay), respectively.

Soluble P-Selectin in Plasma

Plasma concentrations of soluble P-selectin were determined by an ELISA (human soluble P-selectin, R&D Systems). The reference range was 18 to 40 ng/mL.
Plasma Fibrinogen

Plasma fibrinogen levels were determined in a clotting assay by use of a KC 10 coagulometer (Amelung) immediately after collection of the blood samples.11

Flow Cytometry

Blood samples were collected into sodium citrate (final concentration 0.011 mol/L) and diluted 1:50 within 5 minutes in modified Tyrode’s buffer (150 mmol/L NaCl 2.5 mmol/L KCl 12 mmol/L NaHCO3 2 mmol/L MgCl2, 1 mg/mL BSA, 1 mg/mL dextrose; pH 7.4). Platelets were incubated with a saturating concentration of the antibody and 2 µmol/L ADP (final concentration) or PBS for 20 minutes at room temperature. Thereafter, fixation was performed with Cellfix (Becton Dickinson). Blood cells were stored in the dark and analyzed within 12 hours after fixation on a FACScan with Lysis II (Becton Dickinson) software. Studies performed in our laboratory have shown that platelets do not change in forward and sideways scatter and in the fluorescence staining for various surface antigens for up to 72 hours after fixation with Cellfix. Platelets were gated by forward/sideways scatter. These gates were initially established with an anti–GP Ib monoclonal antibody (Dianova). Antibody binding was expressed as percentage of platelets demonstrating a fluorescence intensity that was higher than a predicted marker. All flow cytometry measurements were performed in triplicate.

P-Selectin Expressed on Platelet Surface

P-selectin expressed on the surface of circulating platelets was measured by flow cytometry analysis with a fluorescein isothiocyanate chicken anti-human fibrinogen polyclonal antibody.

Fibrinogen Binding to GP IIb/IIIa Receptors on Platelet Surface

Fibrinogen binding to the platelet surface was measured by flow cytometry analysis with a fluorescein isothiocyanate chicken anti-human fibrinogen polyclonal antibody.

Statistical Analysis

Results of continuous variables are expressed as mean±SD. The significance of differences was tested by χ2 analysis (categoric variables), Student’s t test (continuous variables), and ANOVA (≥2 groups). Significance was defined as P<0.05.

Results

Patients underwent thrombolytic therapy with either alteplase, reteplase, or streptokinase according to the study protocols of RAPID-1, RAPID-2, INJECT, or GUSTO-3. Fifteen patients were treated with 100 mg of alteplase over 180 minutes (group I), and 24 were treated with 100 mg of alteplase over 90 minutes (group II). Only 21 patients could be analyzed in group II because 3 patients had substantially incomplete blood samples. Fifty-two patients received reteplase, reteplase, or streptokinase according to the study protocols of RAPID-1, RAPID-2, INJECT, or GUSTO-3. Fifteen patients were treated with 100 mg of alteplase over 180 minutes (group I), 10 were treated with 100 mg of alteplase over 90 minutes (group II), and 15 were treated with the 10+5-U double-bolus regimen (group III); 2 of these patients had to be excluded from further analysis (1 patient died within the first hour and another patient had substantially incomplete blood samples for other reasons). Fifteen patients were treated with reteplase in the 10+5-U double-bolus regimen (group IV), and another 15 were treated with 15 U of reteplase as a single bolus (group V). Ten patients were treated with 1.5 MU of streptokinase over 1 hour (group VI). Baseline characteristics did not differ significantly between the different treatment groups with regard to age, weight, height, heart rate, and blood pressure at hospital admission, as shown in Table 1.

Platelet Aggregation

Table 2 summarizes the results of the platelet aggregation study. Platelet aggregation is represented by the maximal
slope of the platelet aggregation curve after stimulation with 2 μmol/L ADP.

At baseline, patient groups did not show significant differences in platelet aggregation. All patient groups showed a decrease in platelet aggregation at 1 hour after initiation of thrombolytic treatment. In patients treated with 10+10-U of reteplase (group III) or with streptokinase (group VI), 1-hour levels were significantly lower than at baseline (group III: $-19.4\%$, $P<0.01$; group VI: $-34.8\%$, $P<0.01$).

At 2 hours after the start of therapy, platelet aggregation was still reduced in all patient groups. In patients treated with 10+10-U of reteplase (group III: $-19.4\%$, $P<0.01$) or with streptokinase (group VI: $-26.6\%$, $P<0.05$), levels were still significantly lower than at baseline.

At 12 hours after the start of therapy, platelet aggregation tended to return toward pretreatment levels in all patient groups. Both alteplase patient groups (groups I and II) and the reteplase groups (groups III, IV, and V) showed levels similar to baseline. In streptokinase-treated patients (group VI), platelet aggregation still tended to be lower than at baseline.

When the 3 clinically most important thrombolytic regimens (accelerated alteplase [group II], reteplase 10+10-U double bolus [group III], and conventional streptokinase [group VI]) were compared, marked differences in platelet aggregation could be detected (Table 2). At 1 hour after initiation of thrombolytic therapy, the differences between accelerated alteplase (group II) and reteplase 10+10-U (group III) were significant ($P<0.05$), with lower levels for reteplase. Platelet aggregation was even more decreased in streptokinase-treated patients (group VI), with a significant difference compared with alteplase ($P<0.05$) but not compared with reteplase treatment. After 2 hours, a similar pattern could be detected. Platelet aggregation showed the highest levels in the patients treated with accelerated alteplase (group II), with significant differences compared with reteplase 10+10-U (group III, $P<0.01$) and with streptokinase (group VI, $P<0.01$).

### Plasma Fibrinogen

Table 3 summarizes plasma fibrinogen levels. Assessment of plasma fibrinogen was incomplete in groups II and III; hence, statistical analysis was not performed with these data. It has been shown that plasma fibrinogen decreased to 62% of baseline levels 90 minutes after initiation of thrombolytic therapy with accelerated alteplase.\(^{12}\) Thus, fibrinogen levels of the 6 patients investigated in group II seem to be representative.

At baseline, fibrinogen levels were similar in all treatment groups. However, after 2 hours, there seemed to be differences between patient groups: As expected, the decrease in fibrinogen levels was most pronounced with streptokinase, less with reteplase, and least with alteplase.

### TAT, F1 and F2, and ATIII

Table 4 summarizes characteristic parameters for thrombin activity in the 3 clinically most relevant subgroups: accelerated alteplase (group II), 10+10-U double-bolus reteplase (group III), and streptokinase (group VI). Circulating thrombin levels are represented by TAT concentrations. Baseline and follow-up levels were not significantly different between treatment groups. All groups showed highly significant increases in TAT levels at 1 and 2 hours versus baseline. At 12 hours, TAT levels returned toward pretreatment levels but were still significantly elevated.

Prothrombin fragment (F1 and F2) levels, which indicate the rate of thrombin generation, showed a pattern nearly parallel to that of TAT levels. No significant differences between treatment groups were observed at baseline. At 2 hours, F1 and F2 levels reached maximal levels. At 12 hours, F1 and F2 levels decreased toward pretreatment levels.

ATIII was determined in patients enrolled in the RAPID-2 trial (16 in the alteplase group and 15 in the reteplase group). Plasma levels were not significantly different between alteplase and reteplase treatment groups. ATIII decreased from pretreatment (alteplase: 88.9%±8.2; reteplase: 87.8%±8.1) to 2 hours after start of therapy.
P-selectin in plasma may originate from platelets or endothelial cells, but in the case of platelet activation, elevated P-selectin levels could be expected. Soluble P-selectin was determined in 21 patients treated with accelerated alteplase (group II), 50 patients treated with reteplase 10·10 U double bolus (group III), and 10 patients treated with streptokinase (group VI). No significant differences could be detected in the time course or between treatment groups. Average P-selectin levels were 52.0 ± 20.3 μg/mL at hospital admission, 52.5 ± 19.7 μg/mL at 2 hours, and 53.07 ± 21.3 μg/mL at 12 hours (Table 5).

Platelet surface-membrane–associated P-selectin was measured with and without stimulation of platelets with ADP (final concentration of 2 μmol/L) in 22 patients enrolled in GUSTO-3 (8 patients in the accelerated alteplase group and 14 in the reteplase group; see Figure 1). Levels without stimulation represent the status of platelet activity, and levels after stimulation indicate the capacity of platelets to be activated and secreted. Neither stimulated or unstimulated levels differed significantly between patient groups. Unstimulated P-selectin did not change significantly in the first 12 hours in either group. Stimulated P-selectin decreased slightly during and after thrombolytic therapy, reaching significance after 12 hours compared with baseline in the reteplase group.

Fibrinogen Binding to GP IIb/IIIa Receptors on Platelet Surface
Fibrinogen binding to the GP IIb/IIIa receptor on the platelet surface was determined with and without stimulation of platelets with ADP (final concentration of 2 μmol/L) in 22 patients enrolled in GUSTO-3 (8 in the alteplase group and 14 in the reteplase group; see Figure 2). Unstimulated fibrinogen binding did not differ significantly between patient groups or between time points. Capacity of fibrinogen binding, determined by assessment of fibrinogen binding in flow cytometry after stimulation of platelets by 2 μmol/L ADP, decreased significantly during thrombolysis, with a trend to lower levels with reteplase.

Discussion
Platelet aggregation is influenced by thrombolytic therapy for acute myocardial infarction. Different responses of platelet aggregation to various thrombolytic agents and regimens may contribute to success and complication rates of different thrombolytic regimens. Previous studies assessing platelet function during thrombolytic therapy have shown contradictory results. Some authors suggest that platelets are activated during myocardial infarction and thrombolysis.13–16 These

![Figure 1](http://circ.ahajournals.org/)

*P<0.05 vs 0 h.

![Figure 2](http://circ.ahajournals.org/)

*P<0.05 vs 0 h; **P<0.01 vs 0 h.
studies are mainly based on analysis of arachidonic acid–pathway metabolites or other markers for cell activation in a small number of patients or on in vitro experiments, but not on assessment of platelet aggregation. More recently, flow cytometry experiments showed activation of GP IIb/IIIa within 72 hours after initiation of thrombolytic treatment with alteplase or streptokinase. Other authors, who assessed platelet function during thrombolytic therapy, postulated that a possible platelet-activating effect may be superposed by antiaggregatory effects during thrombolyis. These studies were performed in a small number of patients or after in vitro incubation with thrombolytic agents.

In the present study, a total of 126 patients, recruited in 1 center, who were enrolled in 4 multicenter trials that evaluated alteplase, reteplase, and streptokinase regimens in patients with acute myocardial infarction were investigated. To the best of our knowledge, this is the first study to investigate platelet aggregation in patients with acute myocardial infarction treated by different reteplase regimens. Our results indicate that platelet aggregation is attenuated during and in the first 2 hours after thrombolytic therapy.

When the 3 clinically relevant regimens were compared, the decrease of platelet aggregation was most pronounced with streptokinase, least with 10+10-U double-bolus reteplase, and least with accelerated alteplase. The differences in platelet aggregation between accelerated alteplase and its deletion mutant, reteplase, were significant in the early phase after initiation of therapy. The binding of fibrinogen to platelet surface receptor GP IIb/IIIa, which is the underlying mechanism of platelet aggregation, showed a similar pattern, with significant attenuation during thrombolytic treatment and a trend to lower levels during treatment with reteplase 10+10 U compared with accelerated alteplase, as determined by flow cytometry. Plasma fibrinogen seemed to be degraded to a different extent as well. As expected, fibrinogen levels were decreased to the lowest level with streptokinase, followed by reteplase, and finally alteplase, which agrees with reported differences in the fibrinogen specificity of the agents used. Both effects (impaired fibrinogen binding to platelet GP IIb/IIIa and decreased fibrinogen plasma levels) may well contribute to the observed platelet aggregation effects. We did not observe activation of platelets, reflected by an increase of surface-associated P-selectin in unstimulated platelets or of soluble P-selectin in plasma during the first 12 hours.

Thrombin generation, reflected by levels of prothrombin fragments F1 and F2, is enhanced after initiation of thrombolytic therapy. Highly significant increases in F1 and F2 concentrations could be detected in all treatment groups in the first 2 hours after the start of therapy. Plasma concentrations of thrombin, represented by TAT levels, showed a parallel pattern to thrombin generation, with no significant differences between thrombolytic regimens. ATIII levels decreased during thrombolytic therapy. This may be explained either by higher consumption because of increasing thrombin levels or by plasmin-induced degradation of antithrombin III. Obviously, enhanced thrombin generation during thrombolysis did not result in detectable platelet activation during the first 12 hours. This may be due to the adjunctive therapy with aspirin and heparin.

Because we investigated the early phase after thrombolysis, platelet activation that occurs >12 hours after therapy cannot be excluded and in fact appears likely when the results of Gurbel et al24 are taken into consideration. In that smaller study, treatment with double-bolus reteplase (10+10 U) resulted in a nonsignificant trend toward lower platelet aggregation at 3 and 6 hours than treatment with accelerated alteplase. In contrast, at 24 hours after initiation of thrombolytic therapy, a significant increase in platelet aggregation was observed in the reteplase group compared with the alteplase group. In GUSTO-3, mortality rates were similar during treatment with accelerated alteplase or the reteplase 10+10-U double-bolus regimen in spite of higher early patency rates after reteplase treatment in the angiographically controlled RAPID-1 and RAPID-2 trials. The present data suggest that this phenomenon cannot be explained by platelet activation within the first 12 hours followed by a higher reocclusion rate during therapy with reteplase.

References


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