High Levels of Platelet Inhibition With Abciximab Despite Heightened Platelet Activation and Aggregation During Thrombolysis for Acute Myocardial Infarction

Results From TIMI (Thrombolysis In Myocardial Infarction) 14

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Background—We evaluated platelet activation and aggregation in patients with acute myocardial infarction (AMI) treated with thrombolytic therapy alone or with reduced-dose thrombolysis and concomitant abciximab.

Methods and Results—The study was performed in 20 control subjects and 51 patients with AMI before and after reperfusion with either alteplase or reteplase or reduced doses of these agents with concomitant abciximab. Platelet activation was assayed by platelet surface expression of P-selectin. Turbidometric platelet aggregation in response to ADP was measured in patients before thrombolytic therapy and 90 minutes and 24 hours after the beginning of thrombolytic therapy. P-selectin expression was greater at baseline in patients than normal control subjects (30.4% versus 9.8%, \( P < 0.0001 \)) but was identical between the 2 groups after stimulation with ADP (64.4% versus 69.3%, \( P = 0.37 \)). However, at 24 hours, basal P-selectin expression declined in patients \( (P=0.0025 \text{ versus baseline}) \), whereas ADP-stimulated P-selectin expression was lower in patients than in control subjects \( (48\% \text{ versus } 69\%, \ P=0.0004 \) ).

When combined with reduced doses of either alteplase or reteplase, abciximab achieved 91% and 83% inhibition of 5 and 20 \( \mu\text{mol/L} \) ADP–induced platelet aggregation, which decreased to 46% and 40%, respectively, at 24 hours.

Conclusions—Platelet activation and aggregation are heightened in the setting of thrombolysis for AMI. Despite this enhanced level of platelet activation, abciximab, combined with a reduced-dose thrombolytic, inhibited platelet aggregation similarly to the level reported in elective settings. (Circulation. 2000;101:2690-2695.)

Key Words: myocardial infarction • abciximab • thrombolysis • platelets

Despite important reductions in mortality among patients with acute myocardial infarction (AMI), thrombolytic therapy is still characterized by failure to restore full arterial flow in 45% of patients and mortality rates of 7% to 10%.1 Evidence from several sources indicates that increased levels of platelet activation and aggregation play important roles in the resistance of occlusive arterial thrombi to fibrinolytic therapy.2–9 Recent clinical studies have provided promise that therapy using a combination of a thrombolytic agent and an antagonist of platelet glycoprotein (GP) IIb/IIIa can increase the rate at which arterial patency is restored.10,11 Studies in both canine and primate models of thrombolysis and reocclusion have indicated that blockade of \( \sim80\% \) of platelet surface GP IIb/IIIa with monoclonal 7E3 is required to effect a significant and sustained improvement in arterial patency. This level of blockade corresponded to inhibition of ADP-induced platelet aggregation by \( \sim80\% \) of baseline levels.12,13 Whether this degree of blockade can be attained safely in the setting of AMI has not been fully explored. Doses of platelet GP IIb/IIIa antagonists in current clinical use have been selected on the basis of pharmacodynamic studies in patients undergoing elective percutaneous coronary...
interventions. Although the enhanced state of platelet activation reported in patients undergoing thrombolysis for AMI might be expected to limit the degree to which “standard” doses of GP IIb/IIIa inhibitors inhibit platelet aggregation, data obtained thus far in patients with AMI have not been conclusive.

Accordingly, the purpose of the present study was to evaluate the effects of thrombolytic therapy with and without abciximab on platelet activation and aggregation in the setting of AMI.

**Methods**

**TIMI 14 Study**

TIMI 14 was a dose-ranging trial comparing different reperfusion regimens and comprising 888 patients randomized to abciximab alone, alteplase (tPA) alone, or abciximab combined with reduced-dose tPA or streptokinase and 300 patients randomized to reteplase (rPA) alone or abciximab combined with reduced dose rPA. Patients were treated with aspirin (150 to 325 mg orally or 250 to 500 mg IV). Patients treated with tPA or rPA alone received weight-adjusted heparin (bolus, 70 U/kg; infusion, 15 U · kg⁻¹ · h⁻¹), whereas those treated with regimens containing abciximab and tPA or rPA received heparin doses of either 60-U/kg bolus and 7-U/kg · h⁻¹ infusion or 30-U/kg bolus and 4-U · kg⁻¹ · h⁻¹ infusion. Angiography of the infarct-related artery was performed at 90 minutes.

**Study Patients**

A total of 51 patients were enrolled in the platelet substudy at 6 sites in the United States, United Kingdom, and Belgium between March 1997 and January 1999. After randomization in TIMI 14, patients were enrolled consecutively, provided that the investigator thought that substudy-mandated laboratory determinations could be performed expeditiously. Patients participating in the platelet substudy received 1 of 9 reperfusion regimens (Table 1). An additional 10 healthy control subjects were recruited among study personnel and 10 noncardiac patients. All subjects were studied after informed consent was given and under protocols approved by their human studies review boards.

**Platelet Activation Studies:**

**Membrane-Bound P-Selectin**

In North American subjects (n=31), platelet activation was assessed at baseline and 24 hours by measurement of the percentage of platelets expressing membrane-bound P-selectin. All samples were processed immediately after phlebotomy. Platelet activation was measured in blood samples fixed with 2% formaldehyde immediately without agonist or after a 5-minute incubation with 0.1, or 5 μmol/L ADP at ambient temperature. Fixed samples were refrigerated and shipped overnight to a core laboratory for flow cytometry. Samples from the control subjects were processed similarly. At the core laboratory, samples were washed with PBS and then labeled with saturating amounts of fluorescein-conjugated CD 41 and biotinylated CD 62P monoclonal antibodies as previously described. Platelets were identified with CD41 monoclonal antibody (specific for GP IIb), which labeled all platelets. The percentage of P-selectin–positive platelets was determined by reference to a negative control from which the CD62P monoclonal antibody had been omitted.

**Platelet Aggregation Studies**

Platelet aggregation was assessed in samples collected at baseline and 90 minutes and 24 hours after initiation of thrombolysis. Blood samples were withdrawn through a 19-gauge needle and were analyzed within 1 hour of collection by use of the turbidimetric technique of Born. Blood samples were drawn into citrated tubes (3.8%) and centrifuged at 160g for 12 minutes at room temperature to form platelet rich-plasma according to a standardized protocol. Platelet counts were adjusted to 250 000±50 000 per mm³ with platelet-poor plasma. Platelet aggregation was induced by final concentrations of 5 and 20 μmol/L ADP (Chronolog). Aggregation studies were performed with Chronolog (n=6) and BioData (n=45) aggregometers. Aggregation curves were recorded for 4 minutes, overread by a core laboratory investigator blinded to treatment assignment, and analyzed according to the method of Ruggeri.

**Angiographic and Clinical Event Analysis**

Coronary angiography was performed 90 minutes after initiation of the reperfusion regimen. All angiograms were analyzed in a core laboratory and reported by use of the TIMI flow grading and frame count systems.

**Statistical Analysis**

Continuous variable measurements were expressed as mean±SD. Statistical comparisons were made by χ² analysis for categorical variables and either Student’s t test or Wilcoxon’s ranked-sum test as appropriate for continuous variables. Comparisons were made between continuous variables by use of Spearman’s correlation test.

**Results**

Baseline clinical characteristics and the occurrence of selected clinical events did not differ substantially between patients enrolled in the platelet substudy and those enrolled in the main TIMI 14 study (Table 2).

**Platelet Activation**

Compared with platelets from control subjects, platelets from patients expressed more P-selectin at baseline (30.4% versus 9.8%, P<0.0001) and 24 hours (17.4%, P=0.05 versus control subjects and P=0.0025 versus baseline). Platelet stimulation with increasing concentrations of ADP led to progressive increases in membrane-bound P-selectin (Figure 1). However, platelets from patients at 24 hours expressed less membrane-bound P-selectin after ADP stimulation than did those obtained at baseline or those obtained from normal control subjects. P-selectin expression at 24 hours was not different in abciximab-treated patients compared with those receiving thrombolytic therapy only (Figure 2). P-selectin expression was not different among patients who received ticlopidine after the 90-minute angiogram (n=8) compared with patients who did not receive ticlopidine.

**Platelet Aggregation**

There were no differences in platelet aggregation between patients studied with BioData or Chronolog aggregometers.

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**Table 1. Reperfusion Regimens**

<table>
<thead>
<tr>
<th>Lytic Dose</th>
<th>Thrombolytic Dose</th>
<th>Abciximab Bolus Dose, mg · kg⁻¹ · h⁻¹</th>
<th>Total (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tPA</td>
<td>100 mg</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>65 mg (1)</td>
<td>0.25</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>50 mg (15)</td>
<td>0.25</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>35 mg (2)</td>
<td>0.30</td>
<td>8</td>
</tr>
<tr>
<td>rPA</td>
<td>10+10 U</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>10+5 U (5)</td>
<td>0.25</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>5+5 U (8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are numbers of patients.
TABLE 2. Baseline Patient Characteristics and Selected Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Platelet Substudy</th>
<th>TIMI 14</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=51)</td>
<td>(n=888)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>59±10</td>
<td>58±11</td>
<td>0.59</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>65.6</td>
<td>77.5</td>
<td>0.12</td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>15.6</td>
<td>12.4</td>
<td>0.59</td>
</tr>
<tr>
<td>Prior MI, %</td>
<td>9.3</td>
<td>12.5</td>
<td>0.60</td>
</tr>
<tr>
<td>Anterior MI, %</td>
<td>31</td>
<td>38</td>
<td>0.42</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78±18</td>
<td>80±15</td>
<td>0.60</td>
</tr>
<tr>
<td>White race, %</td>
<td>88</td>
<td>90</td>
<td>0.69</td>
</tr>
<tr>
<td>Prior aspirin use, %</td>
<td>9.4</td>
<td>19.5</td>
<td>0.15</td>
</tr>
<tr>
<td>Platelet count, ×1000/mm³</td>
<td>274±82</td>
<td>247±71</td>
<td>0.04</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.99±0.19</td>
<td>1.02±0.2</td>
<td>0.58</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45±4</td>
<td>44±4</td>
<td>0.21</td>
</tr>
<tr>
<td>Time to treatment, h</td>
<td>4.7±4.8</td>
<td>4.1±4.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Death within 30 d, %</td>
<td>0</td>
<td>3.9</td>
<td>0.26</td>
</tr>
<tr>
<td>Reinfarction within 30 d, %</td>
<td>3.1</td>
<td>2.5</td>
<td>0.81</td>
</tr>
<tr>
<td>Death, MI, or urgent revascularization within 30 d, %</td>
<td>38</td>
<td>34</td>
<td>0.71</td>
</tr>
<tr>
<td>Major hemorrhage, %</td>
<td>9.4</td>
<td>6.5</td>
<td>0.53</td>
</tr>
</tbody>
</table>

For patients receiving tPA or rPA, inhibition to 5 μmol/L ADP at 90 minutes was significantly reduced relative to baseline (P=0.02), indicating an increase in platelet aggregability after thrombolytic treatment. There were no differences in platelet aggregation between patients treated with full-dose tPA or rPA (Table 3). Among patients treated with abciximab 0.25-mg/kg bolus and 0.125-μg · kg⁻¹ · min⁻¹ infusion, no differences in platelet aggregation were observed at 90 minutes or 24 hours after thrombolysis between patients receiving reduced doses of either tPA or rPA (Figure 3).

When combined with reduced doses of either tPA or rPA, both bolus doses of abciximab (0.25 and 0.3 mg/kg) achieved >80% inhibition of platelet aggregation at 90 minutes. Sustained platelet inhibition persisted for ≥24 hours after abciximab therapy was begun (Figure 4). For the 8 patients treated with the higher abciximab bolus (0.3 mg/kg) and reduced-dose tPA, there was less variability of the degree to which platelet aggregation was inhibited. Inhibition of platelet aggregation was >90% in 7 of these 8 patients (86%) compared with only 5 of 21 (24%) of those treated with the 0.25-mg/kg abciximab bolus.

**Angiographic Findings**

No significant correlation was observed between P-selectin expression (at baseline or 24 hours) and either TIMI flow grades or the corrected TIMI frame count. Similarly, there was no correlation between the degree of inhibition of platelet aggregation and angiographic assessments of vessel patency. TIMI flow and frame counts did not differ between the small number of patients in whom platelet aggregation was inhibited ≤80% and most patients in whom inhibition was >80%.

**Clinical Outcomes**

There was no evidence that greater levels of inhibition of platelet aggregation were associated with decreased event rates, whereas they did appear to be associated with higher rates of bleeding (Table 4).

**Discussion**

The dynamic balance between the fibrinolytic system and platelet function is an important determinant of whether reperfusion after thrombolysis will be successful. The present study provides answers to important questions concerning the degree of platelet inhibition that can be obtained by antagonizing platelet GP IIb/IIIa with abciximab in the setting of thrombolysis. Both platelet activation and aggregation in patients with AMI treated with tPA or rPA were enhanced for ≥24 hours. Despite this heightened state of activation, the same doses of abciximab that reduced periprocedural complications in studies of elective percutaneous coronary interventions were able to inhibit platelet aggregation to levels similar to those previously reported but did not alter markers of platelet activation.

Prior observations had suggested that enhanced platelet activation, as observed in acute coronary syndromes, leads to a requirement for a higher dose of a platelet GP IIb/IIIa antagonist. Activation of platelets with strong agonists induces exteriorization of the contents of α granules to the platelet surface. Among the contents of these granules are GP IIb/IIIa molecules. These granules also contain a substantial store of previously unexpressed GP IIb/IIIa, some of which contains “prebound” fibrinogen. Consequently, P-selectin expression is frequently used as a marker of platelet activation. Indeed, some authors have shown that the
indexes of thrombin activity.28 In a setting such as AMI, which is characterized by elevated speculation that a higher dose of abciximab might be needed 10 U (double mg (bolus); rPA 5 after beginning therapy, according to treatment group. tPA 100 Inhibition of ex vivo platelet aggregation 90 minutes of infusion); ADP.15 This observation led to platelets stimulated ex vivo with thrombin receptor agonist peptide is used. A higher concentration of abciximab was needed to inhibit the aggregation of thrombin receptor agonist peptide compared with ADP.15 This observation led to speculation that a higher dose of abciximab might be needed in a setting such as AMI, which is characterized by elevated indexes of thrombin activity.28 Consequently, 2 bolus doses of abciximab, 0.25 and 0.30 mg/kg, were examined in TIMI 14. There was no evidence that increasing the bolus of abciximab from 0.25 to 0.3 mg/kg increased the number of patients in whom >80% inhibition of aggregation was achieved. However, 7 of the 8 patients treated with the higher abciximab bolus (0.3 mg/kg) had >90% inhibition of platelet aggregation 90 minutes after initiation of reperfusion compared with only 31% of patients treated with the 0.25-mg/kg bolus. Although it is conceivable that the increased degree of inhibition observed with the latter dose might enhance reperfusion even more than the 0.25-mg/kg bolus, data from TIMI 14 indicated that increasing the abciximab dose is associated with higher rates of hemorrhagic complications.11 The present study indicates that even during thrombolysis, profound inhibition of platelet aggregation can be achieved without increasing the dose of abciximab. Whether the number of additional receptors expressed in this clinical setting is relatively small or whether the molar excess of abciximab contained in a bolus dose of 0.25 mg/kg is adequate to block the additional receptors is not known.

Relationship Between Levels of Inhibition and Clinical Indexes
Although TIMI 14 reported a robust improvement in the rate of early reperfusion for reduced-dose thrombolytic therapy combined with abciximab, we were not able to observe a correlation between angiographic indexes of coronary flow and the degree to which platelet aggregation was inhibited. This lack of correlation may be related to the imprecision associated with both turbidimetric platelet aggregation and angiographic assessment of coronary flow. The present study was not designed to provide definitive associations between the degree of inhibition of platelet aggregation and clinical event rates. Nonetheless, higher levels of platelet inhibition were not associated with lower rates of recurrent events. As did other investigators, we observed that bleeding was more frequent among patients with greater degrees of inhibition.27,29 Platelet Desensitization
A novel and important observation from the present study is that the ability of stimulated platelets to express additional membrane-bound P-selectin after ADP stimulation decreased during the first 24 hours after thrombolysis (Figure 1). P-selectin expression did not differ among patients treated with abciximab and reduced-dose thrombolytics and those receiving thrombolytics alone. The explanation for this phenomenon is not clear, but several mechanisms are possible.

First, as a result of exposure to the various biologic agonists operative in the setting of AMI, α granules or their

![Platelet Aggregation 90 mins](image1)

**Figure 3.** Inhibition of ex vivo platelet aggregation 90 minutes after beginning therapy, according to treatment group. tPA=100 mg (bolus +90 minutes of infusion); rPA=10 U+10 U (double bolus); t0.25=reduced-dose tPA (35 to 65 mg)+0.25-mg abciximab bolus; r0.25=reduced-dose rPA (6+5 U, 10+5 U)+0.25-mg abciximab bolus; and t0.3=reduced-dose tPA (50 mg)+0.30-mg/kg abciximab bolus.

![Platelet Aggregation 24 hrs](image2)

**Figure 4.** Inhibition of ex vivo platelet aggregation at 24 hours according to treatment group. Abbreviations as in Figure 3.
contents may become depleted in circulating platelets.\textsuperscript{30,31} Second, shedding or internalization of P-selectin by previously activated platelets is possible, or the most activated platelets may become bound to leukocytes, incorporated into thrombi, or otherwise removed from the circulation. Finally, homologous or heterologous desensitization\textsuperscript{32–34} or fatigue of the intracellular mechanisms responsible for transducing the signal of ADP stimulation because of prolonged in vivo exposure to ADP is also possible. The functional significance of these findings is not clear. However, because platelets form complexes with leukocytes through the interaction of P-selectin with a glycoprotein ligand found on leukocytes, loss of the ability to express P-selectin may affect the ability of platelet-leukocyte complexes to release a variety of inflammatory and mitogenic mediators at the site of plaque rupture and may alter platelet–endothelial cell adhesion.\textsuperscript{34} This finding may in fact reflect a prolonged period of agonist stimulation and may indicate that platelets remain activated for a prolonged period of time after thrombolysis, thus providing a potential explanation for the high frequency of recurrent ischemic events after thrombolysis.

**Study Limitations**

The primary limitation of this study is the small number of patients studied in the individual treatment groups. In addition, some differences in results of platelet aggregation studies may have arisen from technical variations in the performance of turbidimetric aggregometry at multiple sites. Attempts to minimize these effects include using a standardized protocol, training clinical laboratory personnel, providing standardized reagents to each site, and using a blinded core laboratory investigators to review the quality and accuracy of the platelet aggregation tracings. Although turbidimetric ex vivo aggregometry provides a limited view of platelet participation in thrombosis, it is currently the most reproducible and most accepted functional measure of platelet activity. Finally, measurement of platelet aggregation in response to >1 agonist might have allowed a more precise answer to whether the observed reduction in P-selectin expression was related to exhaustion of \( \alpha \) granule stores or to true desensitization.

**Conclusions**

Heightened platelet activation and aggregation in patients with AMI treated with tPA and rPA persist to \( \geq 24 \) hours. “Standard-dose” abciximab, used in conjunction with reduced-dose thrombolytic, achieves \( \geq 80\% \) levels of inhibition 90 minutes after the beginning of therapy with a reduced-dose thrombolytic agent. There is still evidence of a significant antiaggregatory effect at 24 hours. Thus, abciximab appears to be an effective regimen for counteracting the milieu of heightened platelet activation and aggregability in patients treated with thrombolytic therapy.

**Appendix**

**Core Laboratories**

Platelet activation and aggregation review: Maine Medical Center Research Institute, South Portland, Me. Principal investigator: Kenneth A. Ault, MD; technician: Jane Mitchell. Angiographic core laboratory: West Roxbury, Mass. Principal investigator: C. Michael Gibson, MD, MS; quantitative angiography technician: Kathryn Ryan, BS; data manager: Sabina Murphy, MPH.

**Sponsors**

Centocor, Malvern, Pa: Keaven Anderson, PhD; Elliot Barnathan, MD; Richard P. Schwarz, Jr, PhD; and Ann Wang. Eli Lilly, Inc, Indianapolis, Ind: Joel Scherer, MD, Shirley Paddock, RPh, and Kimberly Hadley, BS.

**Data Coordinating Center**

COVANCE, Princeton, NJ. Global program director: Lillian Dampman, PhD; project director: Kevin Vernarec.

**Enrolling Centers (in Order of Enrollment)**

Methodist Hospital and Ben Taub Hospital, Houston, Tex: principal investigator, Neal S. Kleiman, MD; research coordinator, Kelly Maresh, RN. Royal Victoria Hospital, Belfast, UK: co–principal investigators, A.A. Jennifer Adgey and Ian Menown; research coordinators, Bernice Smith and Leslie Swailes. Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium: coinvestigators, Frans Van de Werf and Patrick Coussemé, MD; research coordinator, Patrick Coussemé, MD. Montefiore Medical Center, Bronx, NY: coinvestigators, Mark Greenberg, MD, and Hilfrud Mueller, MD; research coordinators, Joseph Cosico, RN, and Kelly Schneider, RN. John L. McClellan Veteran’s Memorial Hospital, Little Rock, Ark: principal investigator, J. David Talley, MD; research coordinators, Millie Rawert, BSN, and Mindy Dearen. Sarasota Memorial Hospital,
Sarasota, Fla: principal investigator, Martin J. Frey, MD; research coordinator, Holly Taylor.

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References
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