Effect of Thrombolytic Therapy on Platelet Expression and Plasma Concentration of PECAM-1 (CD31) in Patients With Acute Myocardial Infarction

Victor L. Serebruany, Paul A. Gurbel

Abstract—Animal studies have shown that the administration of antibodies against platelet/endothelial cell adhesion molecule-1 (PECAM-1) before reperfusion can reduce infarct size. The purpose of the present study was to define the effects of thrombolytic therapy in acute myocardial infarction (AMI) patients on the platelet expression and plasma concentrations of PECAM-1 at prespecified time points after attempted reperfusion. The plasma concentration and platelet expression of PECAM-1 were determined in 23 AMI patients enrolled in the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO-III) trial before thrombolysis and at 3, 6, 12, and 24 hours thereafter and compared with 22 healthy controls. At baseline, PECAM-1 was expressed significantly more on the platelet surface in the AMI patients than in controls ($P = 0.027$) while soluble PECAM-1 plasma levels were almost identical between groups. There were no significant diurnal variations in both plasma and platelet PECAM-1 levels in controls. A significant decrease in platelet PECAM-1 expression was observed 3 hours after thrombolysis ($P = 0.03$) compared with baseline, followed by a significant increase ($P = 0.004$) in fluorescence intensity later at 24 hours after thrombolysis. Conversely, a significant increase in soluble PECAM-1 was observed 3 hours after thrombolysis ($P = 0.02$), followed by a significant decrease later at 24 hours after attempted reperfusion ($P = 0.03$). The expression of platelet-bound PECAM-1 is increased in AMI patients. Discordantly directed changes in soluble and platelet PECAM-1 after the first 24 hours after thrombolytic therapy may represent redistribution of the whole PECAM-1 pool. Further investigation of the possible role of PECAM-1 and the relationship between its soluble and platelet fractions in AMI are warranted. (Arterioscler Thromb Vasc Biol. 1999;19:153-158.)

Key Words: PECAM-1 | acute myocardial infarction | thrombolysis | humans

Platelet/endothelial cell adhesion molecule-1 (PECAM-1, CD31), a 130-kDa integral membrane glycoprotein (GP) and a member of the immunoglobulin gene superfamily, is found on the surface of platelets and leukocytes and at intercellular junctions of endothelial cells. PECAM-1 is directly involved in the formation of the vascular bed, affects the upregulation of integrin function on leukocytes, and has been implicated as a trigger that regulates leukocyte trafficking through the vessel wall. As an α-granule constituent, PECAM-1 is a distinct, well-defined component of the platelet plasma membrane, with an intracellular distribution identical to that of GP Ib/IIa. Native, resting human platelets express $\approx 8000$ molecules per platelet, whereas thrombin-stimulated platelets exhibit nearly 2-fold expression.

A soluble form of PECAM-1, which is 5 to 10 kDa smaller than platelet-associated PECAM-1, contains a cytoplasmic tail and is encoded by an alternatively spliced mRNA from which the exon containing the transmembrane domain has been removed. Despite the proposed importance of PECAM-1, little is known about its biosynthesis, processing, and turnover on the cell surface.

Limited evidence from in vitro and animal studies show that monoclonal antibodies against PECAM-1 block leukocyte migration and neutrophil accumulation and reduce acute inflammation. Recognizing the role of leukocytes in the pathogenesis of acute coronary thrombosis and the modulation of injury after reperfusion, it is reasonable to expect that anti–PECAM-1 antibodies may diminish infarct size. Indeed, the administration of monoclonal antibodies against PECAM-1 resulted in a significant reduction in infarct size, presumably via blockade of neutrophil accumulation in the myocardium in both rats and cats. These studies suggest that PECAM-1 may be an attractive target for a novel, adjunctive therapeutic approach in the treatment of acute myocardial infarction (AMI).

However, the role of PECAM-1 in acute coronary syndromes has been suggested but never explored. No data are available on platelet PECAM-1 expression and plasma levels in AMI patients; similarly, the effects of thrombolytic therapy are unknown. Moreover, possible diurnal variations in platelet and plasma PECAM-1 levels in healthy controls have not been elucidated. Simultaneous determination of both platelet
and soluble forms could lead to the discovery of helpful correlations between PECAM-1 and arterial patency, the success of thrombolysis, infarct size, reocclusion, or reinfarction.

Thus, the purpose of the present study was to define the immediate, early, and delayed effects of thrombolytic therapy in AMI patients enrolled in the Global Use of Strategies to Open Occluded Coronary Arteries (GUSTO-III) trial on the platelet expression and plasma concentrations of PECAM-1 at prespecified time points after attempted reperfusion and to compare these results with a cohort of healthy controls matched over the same time period at the same time of day.

Methods

This study was approved by the Institutional Review Boards of St Agnes Hospital and Union Memorial Hospital (Baltimore, Md). Informed, written consent was obtained from all participants of the study.

Controls

Ten nonsmoking, nondiabetic subjects (aged 21 to 43 years; 6 men, 4 women) without a history of bleeding disorders, hypertension, and cardiovascular disease and who did not use pharmacological agents for at least 2 weeks before the study were enrolled. All subjects underwent blood sampling after at least 30 minutes of rest and 2 or more hours of fasting. From 12 additional volunteers, blood was drawn 6 times every 4 hours over a 24-hour period to determine any possible diurnal influence and was sampled from an antecubital vein as in the experimental group.

Patients

Twenty-three consecutive patients admitted to the emergency departments of St Agnes Hospital or Union Memorial Hospital between July and December 1996 with a diagnosis of AMI were included. All patients were enrolled in the randomized trial of reteplase (n=13) versus accelerated alteplase (n=10) for treatment of AMI (GUSTO-III trial).13 The inclusion criteria have been previously reported.11 In summary, patients of any age who presented within 6 hours of symptom onset with >30 minutes of continuous symptoms of AMI and who, on a 12-lead ECG, had demonstrated at least a 1-mm ST-segment elevation in 2 or more limb leads, at least a 2-mm ST-segment elevation in 2 or more contiguous precordial leads, or bundle branch block were included in this trial. Patients were excluded if they had a history of bleeding diathesis, stroke, major surgery, or significant trauma in the previous 6 weeks and a blood pressure reading >200/110 mm Hg. Patients randomized to reteplase therapy received 2 10-MU boluses given 30 minutes apart. Those randomized to alteplase received an accelerated dosing regimen: a 15-mg bolus, then 0.75 mg/kg over 30 minutes, and 0.50 mg/kg over 1 hour. During baseline sampling, every patient received 325 mg aspirin and at least 5000 U intravenous heparin. After administration of the thrombolytic therapy, all patients received a continuous infusion of heparin for the first 24 hours as recommended in the GUSTO-III protocol. Blood samples for ELISA and for flow-cytometric studies were taken at prespecified intervals: in the emergency department immediately before administration of the thrombolytic therapy, in the coronary care unit at 3, 6, and 12 hours; and finally at 24 hours thereafter. To avoid possible observer bias, blood samples were coded and blinded. Sampling procedures, ELISA, and flow-cytometric studies were performed by individuals unaware of the protocol.

Time Course and Exclusion of Blood Samples

The schedules for blood drawing, sample preparation, and processing were critical issues of the study design and were monitored by an independent observer. The actual timing of blood collection for the baseline sample was 9.5±1.4 minutes before the start of thrombolytic therapy, 174.6±24.2 minutes for the 3-hour sample, 371.1±24.2 minutes for the 6-hour sample, 709.4±17.8 minutes for the 12-hour sample, and 1402.9±18.8 minutes for the 24-hour sample. Samples were processed within 1 hour after blood drawing. Four patients did not complete the protocol at various time points. The reasons for early termination were patient transfer for emergency coronary angioplasty (3) and inability to obtain a blood sample (1). Twenty-three baseline samples, 22 samples collected at 3 hours, 20 samples collected at 6 hours, 20 samples collected at 12 hours, and 19 samples collected at 24 hours were included in the study analysis.

Soluble PECAM-1

Platelet-poor plasma was obtained by centrifugation of whole blood at 4°C in a Labofuge at 3000g for 10 minutes. Samples were stored at −80°C before final determinations. An ELISA for PECAM-1 (Bender MedSystems) was used according to standard techniques. Each sample was measured in triplicate, and the overall intra-assay coefficient of variation was 2.1±0.3%.

Platelet-Bound PECAM-1

Flow-cytometry procedures have been previously described in detail.14,15 In brief, venous blood (8 mL) was collected in a plastic tube containing 2 mL acid-citrate-dextrose (ACD) and mixed well. The blood-ACD mixture was centrifuged at 1000 rpm for 10 minutes at room temperature. The upper ½ of the platelet-rich plasma (PRP) was then collected and adjusted to pH 6.5 by adding ACD. The PRP was then centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and the platelet pellet gently resuspended in 4 mL of the washing buffer (10 mmol/L Tris-HCl, 0.15 mol/L NaCl, and 20 mmol/L EDTA, pH 7.4). Platelets were washed 4 times in the washing buffer and an additional 4 times in Tris-buffered saline (10 mmol/L Tris and 0.15 mol/L NaCl, pH 7.4). One portion of washed platelets was incubated with 5 µL FITC-conjugated antibodies in the dark at 4°C for 30 minutes, and 1 part remained unstained and served as a negative control. Surface antigen expression was determined with monoclonal murine anti-human antibodies to CD31 (PECAM-1; PharMingen, San Diego, Calif). After incubation, the cells were washed 3 times with Tris-buffered saline and resuspended in 0.25 mL of 1% paraformaldehyde. Samples were stored at 4°C and analyzed on a Becton-Dickinson FACScan flow cytometer with a laser output of 15 mW, an excitation wavelength of 488 nm, and emission detection at 530±30 nm. The instrument was calibrated daily with fluorescence beads (CalibRITE; Becton-Dickinson) and measured FITC-conjugated fluorescence intensity. All parameters were obtained by logarithmic amplification to the fourth decimal place. The data were collected and stored in list mode and then analyzed with CELLQuest (version 1.2.2) software.

Statistical Analyses

A post hoc t test comparison using the Bonferroni correction was performed to identify specific differences in soluble PECAM-1 and platelet receptor expression between AMI patients and controls and between different time points within the control and AMI groups. A Mann-Whitney U test was used to analyze nonparametric data. Normally distributed data were expressed as mean±SD, and P<0.05 was considered significant. Differences between individual flow-cytometric histograms were assessed using the Smirnov-Kolmogorov test incorporated in the CELLQuest software.

Results

The clinical characteristics of the AMI patients are shown in Table 1. Table 2 and Figure 1 summarize the data on the soluble and platelet-bound PECAM-1 levels in AMI patients before and after thrombolytic therapy and in healthy controls. Individual data on the diurnal variations in healthy volunteers are plotted for soluble (Figure 2A) and platelet-bound (Figure 2B) PECAM-1. There were no meaningful differences in diurnal variations in healthy volunteers. However, platelet-bound PECAM-1 was slightly higher during the night and early morning hours.
At baseline, the data were almost identical between controls and the AMI group. A significant increase of plasma PECAM-1 was observed 3 hours after thrombolysis ($P<0.02$) and was followed by a significant decrease later at 24 hours ($P<0.03$).

**Platelet-Bound PECAM-1**
At baseline, before any reperfusion strategies were applied, PECAM-1 was expressed significantly more on the platelet surface of the AMI patients compared with controls ($P<0.027$). A significant decrease of platelet PECAM-1 expression was observed 3 hours after thrombolysis ($P<0.03$) when compared with baseline, followed by a significant increase ($P=0.004$) in fluorescence intensity later at 24 hours after thrombolysis.

**Discussion**
The data from the present study describe the time course of soluble and platelet-bound PECAM-1 levels after thrombolytic therapy in patients with AMI. A negative correlation was observed for soluble and platelet-bound PECAM-1 at 3 and 24 hours after thrombolysis. That is, while plasma PECAM-1 increased early after thrombolysis, platelet expression of PECAM-1 diminished at the same time point. Conversely, later after thrombolytic administration, soluble PECAM-1 levels dropped below baseline while platelet expression increased significantly (Figure 1). The present data suggest that plasma levels and platelet expression of PECAM-1 are inversely related to each other during the course of thrombolysis for AMI. Future basic studies are necessary to determine the normal distribution of the human PECAM-1 pool and the factors affecting this adhesion molecule.

This study also demonstrates that the expression and plasma levels of PECAM-1 in the AMI population are heterogeneous. We have observed similar heterogeneity with other platelet-surface receptors, including P-selectin and GP IIb/IIIa. Contrary to expectations, we did not observe marked PECAM-1 activation at baseline in the AMI population when compared with controls. Certain individuals exhibited a 2-fold increase in PECAM-1; however, more than a half of

### Table 1. Characteristics of the AMI Patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AMI Patients (n=23)</th>
<th>Controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>18/5</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>61.1±4.9</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Previous infarction</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Duration between pain onset and blood drawn, min</td>
<td>221.6±98.6</td>
<td></td>
</tr>
<tr>
<td>Concomitant medications</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium-channel inhibitors</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>WBC, ×10^9/mL</td>
<td>8.1±1.9</td>
<td></td>
</tr>
<tr>
<td>RBC, ×10^12/mL</td>
<td>3.86±0.7</td>
<td></td>
</tr>
<tr>
<td>Platelets, ×10^9/mL</td>
<td>230.0±12.6</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>15.3±0.7</td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42.2±4.2</td>
<td></td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.0±0.2</td>
<td></td>
</tr>
</tbody>
</table>

ACE indicates angiotensin converting enzyme; WBC, white blood cell count; and RBC, red blood cell count. Values are numbers of patients with each characteristic.

### Table 2. Soluble and Platelet-Bound PECAM-1 Levels in AMI Patients After Thrombolytic Therapy and in Healthy Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>3 Hours</th>
<th>6 Hours</th>
<th>12 Hours</th>
<th>24 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble PECAM-1</td>
<td>31.72±11.21</td>
<td>41.83±11.56</td>
<td>32.30±6.64</td>
<td>33.24±6.86</td>
<td>25.54±5.85</td>
</tr>
<tr>
<td>Platelet PECAM-1</td>
<td>56.84±17.69</td>
<td>50.13±14.24</td>
<td>51.12±15.67</td>
<td>58.80±17.02</td>
<td>74.96±19.01</td>
</tr>
</tbody>
</table>

Values are mean±SD and are expressed as ng/mL for the soluble form and log fluorescence intensity for platelet expression.

* $P<0.05$ vs corresponding baseline measurement.
† $P<0.05$ vs corresponding measurement between groups.
AMI patients have a platelet expression of PECAM-1 that is within the normal range or lower. Furthermore, the soluble PECAM-1 level was identical between the AMI group and healthy controls.

Platelets have established interactions with both physiological and drug-induced fibrinolysis. For example, platelet α-granules, in addition to containing PECAM-1, incorporate plasminogen activator inhibitor-1 and α2-antiplasmin.

Figure 2. Representative individual plots and means of plasma levels (A) and of platelet-bound PECAM-1 expression (B) in 12 healthy volunteers over 24 hours. Platelet P-selectin is slightly higher during the night and morning hours.

AMI patients have a platelet expression of PECAM-1 that is within the normal range or lower. Furthermore, the soluble PECAM-1 level was identical between the AMI group and healthy controls.
lease of these proteins theoretically could result in reduced thrombolysis. The resistance to arterial opening after thrombolysis has been reported in animal models of platelet-rich coronary thromb.18 Studies supporting the "platelet hypothesis" have demonstrated enhanced reperfusion with concomitant use of antibodies and other antagonists to GP IIb/IIIa.19,20 In addition, it has recently been demonstrated that systemically administered antibodies against GP IIb/IIIa alone, presumably by facilitating platelet disaggregation, can restore infarcted artery reperfusion.21 Nevertheless, the relative contribution of each of the individual platelet-surface receptors to platelet-mediated vessel occlusion in the pathogenesis of AMI remains undefined.

Flow cytometry has been found to be a sensitive, objective, and reproducible method for the detection and measurement of platelet receptors.14,15 A large number of cellular antigens have been demonstrated on platelets by flow cytometry, and their quantification is an objective and accurate method.22 Some of the platelet receptors (eg, GP IIb/IIIa, P-selectin) are relatively well described, and their role in acute coronary syndromes is under thorough investigation, whereas much less is known about other surface GPs, including PECAM-1.

The involvement of PECAM-1 in platelet adhesion and aggregation remains a matter of considerable controversy. Anti-PECAM-1 monoclonal antibodies did not inhibit platelet aggregation or platelet adherence to the extracellular matrix23 and had no effect on platelet aggregate formation after epinephrine-induced activation24 in humans. However, although no effects of PECAM-1 on platelet aggregation have been reported, an important role in aggregation-induced cell signaling has been observed.24 In contrast, PECAM-1 has been implicated in platelet aggregation25 and has been described as an important modulator of platelet function in mice.26 Our data on the diminished platelet expression of PECAM-1 followed by its increase later during thrombolysis is in agreement with the few available observations on the dynamic patterns of the platelet-27 and neutrophil-28 expressed PECAM-1 during cardiopulmonary bypass in humans.

Another meaningful issue is an obvious concern for a possible diurnal variation of PECAM-1 in normal controls, which were described earlier for the fibrinolytic system29 and platelet serotonin transport30 but not for platelet aggregability31 and membrane α2-adrenoceptor expression32 or eicosanoid urinary excretion.33 Diurnal variations are important because there is clear clinical evidence of circadian patterns in myocardial ischemic episodes. In patients with effort angina, the highest activity occurs between 6 AM and noon. This maximum coincides with peaks in the diurnal variation in frequency of AMI, stroke, and sudden death.34,35 Indeed, we found no significant diurnal variations in both soluble and platelet PECAM-1 values; however, slightly higher levels of platelet-bound PECAM-1 expression during the night and early morning hours are in full agreement with the above-mentioned studies.

There are several limitations of the study. The sample size is small and therefore is compromised by low power. Statistical differences between and within groups in the soluble form and in surface platelet receptor expression could possibly be revealed in a larger sample of patients. It will be important in future studies to determine possible interactions between PECAM-1 and more-explored platelet antigens like GP IIb/IIIa and P-selectin and to develop specific anti-PECAM-1 strategies in patients with acute coronary syndromes. In addition to the observed differences in PECAM-1, clinical characteristics such as use of antecedent aspirin and the timing of thrombolytic agent delivery may influence the results.

In conclusion, PECAM-1 plasma concentrations are dynamic after thrombolytic therapy in AMI patients and exhibit an early rise followed by a slow decline. The mechanism of the above observation is unknown but may be related to myocardial reperfusion. An enticing speculation is that it is derived from the ischemic vascular bed after reperfusion. Platelet PECAM-1 is increased at baseline in the AMI population, declines early after reperfusion, and is followed by a second phase of enhanced expression. This phenomenon, observed with other platelet antigens, may indicate delayed platelet activation after thrombolysis.

The determination of the role of less-studied platelet and endothelial receptors in patients with AMI cannot be overstated. Given the predominance of PECAM-1 on the endothelium, its proposed importance in transendothelial migration of leukocytes, and the role of leukocytes and platelets in coronary thrombosis, further investigation of the relation between soluble and platelet PECAM-1 fractions in myocardial ischemia/reperfusion is warranted.

Acknowledgments
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References
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