Pharmacodynamic Profile of Short-term Abciximab Treatment Demonstrates Prolonged Platelet Inhibition With Gradual Recovery From GP IIb/IIIa Receptor Blockade

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Background—The glycoprotein (GP) IIb/IIIa receptor antagonist abciximab is approved for use in high-risk percutaneous coronary interventions. The purpose of the present study was to establish the pharmacodynamic profile and platelet-bound life span of abciximab.

Methods and Results—The pharmacodynamics of abciximab (inhibition of ex vivo platelet aggregation and GP IIb/IIIa receptor blockade) were measured in 41 individuals who were randomized to receive a 0.25-mg/kg bolus and a 12-hour infusion of either 10 µg/min (EPIC regimen) or 0.125 µg · kg⁻¹ · min⁻¹ (EPILOG regimen) of the antiplatelet agent. At extended times, the amount and distribution of platelet-bound abciximab were monitored by flow cytometry. The EPIC and EPILOG infusion regimens exhibited equivalent blockade of both GP IIb/IIIa receptors and platelet aggregation throughout the duration of abciximab treatment. Flow cytometry revealed a single, highly fluorescent platelet population during treatment, consistent with complete saturation and homogeneous distribution of abciximab on circulating platelets. For 15 days after treatment, the fluorescence histograms remained unimodal with gradually diminishing fluorescence intensity, indicating decreasing levels of platelet-bound abciximab. At 8 and 15 days, which exceeds the normal circulating life span of platelets, median relative fluorescence intensity corresponded to 29 100 (29% GP IIb/IIIa receptor blockade) and 13 300 (13% GP IIb/IIIa receptor blockade) abciximab molecules bound per platelet, respectively.

Conclusions—These results are consistent with continuous reequilibration of abciximab among circulating platelets and may explain the gradual recovery of platelet function and long-term prevention of ischemic complications by abciximab after coronary intervention. (Circulation. 1998;97:1680-1688.)

Key Words: drugs n pharmacology n receptors

The EPIC and EPILOG studies established that the antithrombotic agent abciximab (commercial name, ReoPro) reduces the risk of ischemic complications in patients undergoing percutaneous coronary intervention.1,2 Abciximab is a murine-human chimeric Fab fragment of the monoclonal 7E3 IgG1 that was derived from immunization of a mouse with human platelets. Abciximab inhibits platelet function by engaging the GP IIb/IIIa (α₃β₃) receptor and thereby prevents the binding of fibrinogen and von Willebrand factor to activated platelets. Abciximab also binds with equivalent affinity to another integrin, the vitronectin (αᵥβ₃) receptor, which is present on platelets, vascular endothelial cells, and smooth muscle cells.4 This receptor shares the same β₃-subunit as GP IIb/IIIa.5 Abciximab also has anticoagulant properties; it inhibits platelet-mediated, tissue factor–dependent thrombin generation in vitro.6 Even though the mechanisms by which abciximab modulates platelet function have been extensively investigated both in vitro6-12 and in vivo,13-18 the pharmacological characteristics that make abciximab such a highly effective antithrombotic agent are not completely understood. Therefore, the objective of the present study was to monitor the pharmacodynamics of abciximab in subjects who received a 0.25-mg/kg bolus followed by a 12-hour infusion of either 10 µg/min (EPIC regimen) or 0.125 µg · kg⁻¹ · min⁻¹ (EPILOG regimen) of the antiplatelet agent. The effects of abciximab on platelet function were monitored by measurement of both GP IIb/IIIa receptor blockade and ex vivo light transmittance platelet aggregation both during and after abciximab therapy. In addition, flow cytometry was used to monitor the lifespan and distribution of abciximab on the circulating platelet population during and after treatment. In contrast to radiometric measurements of receptor binding that yield an average number of available abciximab surface binding sites, flow cytometry also reports the distribution of these sites within the cell population. In addition, these analyses were extended to quantify the
amount of circulating platelet-bound abciximab at 8 and 15 days after treatment.

Methods

Chemicals and Reagents
Abciximab was manufactured by Centocor Inc. Polyclonal rabbit anti-abciximab reagent was generated by immunization of rabbits with murine 7E3 Fab. The IgG component was isolated from the serum of rabbits immunized with murine 7E3 Fab by protein A-Sepharose (Pharmacia). The rabbit anti-abciximab–specific components were further purified from the remaining IgG fraction by affinity chromatography on an immobilized chimeric 7E3 Fab(ab')₂ column. Except where noted, all other chemicals were purchased from commercial sources and used without further purification.

Study Population and Enrollment Criteria
The trial was a single-center, open-label study conducted at the National Medical Research Corp in Hartford, Conn. The study comprised 41 volunteer subjects (24 men and 17 women who were not of child-bearing potential) between the ages of 21 and 80. Individuals who were administered ticlopidine 1 month before enrollment were excluded from the study. All subjects were given an informed consent. The study was approved by the institutional review board of the National Medical Research Corp, and all patients gave written consent.

Blood Collection for Pharmacodynamic Measurements
Predose and postabciximab bolus blood samples (0.5- through 24-hour time points) were drawn through the cap of the indwelling catheter. The remaining samples were drawn by direct venipuncture through an 18-gauge needle. Blood was collected into polypropylene syringes containing 1/100 volume of 40% trisodium citrate. The indwelling catheter was placed in the antecubital vein of the arm opposite the one in which the drug was being administered. Five milliliters of blood was drawn from the indwelling catheter and discarded before the blood samples were drawn. After each blood draw, the catheters were flushed with 3 mL of normal saline solution and refilled every 2 to 3 hours with saline until their removal.

Platelet Counts
Whole-blood platelet count measurements (baseline, 24 hours, and 3, 8, 15, and 28 days after treatment) were determined on samples collected into vacuum tubes containing EDTA.

Pharmacodynamic Measurements

Platelet Aggregation
The inhibition of platelet aggregation was evaluated on PRP samples by the turbidimetric method as previously described. The extent of platelet aggregation was quantified as the maximum change in light transmittance within 4 minutes after addition of agonist. For each sampling time, the percent baseline aggregation was determined by the following calculation:

\[
\frac{(\text{Maximum Change in Light Transmittance of Test Sample})}{(\text{Maximum Change in Light Transmittance of Baseline Sample})} \times 100\% 
\]

GP IIb/IIIa Receptor Blockade Assay
The total number of baseline abciximab receptors and the degree of GP IIb/IIIa receptor blockade at postabciximab treatment times were quantified by the radiometric method of Coller. The percent GP IIb/IIIa receptor blockade was calculated as follows:

\[
\frac{[\text{Baseline (Total) GP IIb/IIIa Receptors}] \sim \{\text{Post-Injection (Unoccupied) GP IIb/IIIa Receptors}\}}{[\text{Baseline (Total) GP IIb/IIIa Receptors}] \times 100\%}
\]

Flow Cytometry Analysis

Ex Vivo Flow Cytometric Studies
All incubations were performed at room temperature. PRP was placed in an amber-colored Eppendorf tube (Brinkmann Instruments). FITC–rabbit abciximab antibody (final concentration, 40 μg/mL) was added to the PRP, and the sample was incubated for 5 minutes. The samples were then diluted with an equal volume of 1% formalin (Aldrich Chemical Co) in PBS, pH 7.4, and incubated for 5 minutes to eliminate any in vitro equilibration of abciximab. They were then diluted with an equal volume of quenching solution (50 mmol/L Tris, 10 mmol/L glycine, 150 mmol/L NaCl; pH 7.4), immediately stored in the dark at 4°C, and shipped on ice within 24 to 48 hours of preparation. Flow cytometric analyses were performed on a Becton Dickinson FACScan, and data were collected on 5000 events. For each sample, single, intact platelets were identified by the forward- versus side-scatter profile, and a gate was set around the single-cell population to eliminate debris and platelet microaggregates. If <50% of collected events fell within this gate, the sample was not acceptable, and these data were excluded from statistical analysis. Of the 285 samples that were analyzed, 15 were excluded on the basis of this criterion.
Calibration of Fluorescence Intensity
PRPs from normal human donors (n = 3) were incubated with varying concentrations of either ¹²⁵I-labeled abciximab or an equivalent concentration of unlabeled drug. All samples were incubated for 30 minutes at room temperature. The amount of ¹²⁵I-abciximab bound to platelets was quantified as described above. For the samples treated with unlabeled abciximab, prostaglandin E₁ (100 nmol/L) and apyrase (0.1 U/mL) were added to the PRP, and the platelets were centrifuged at 500 g for 10 minutes. The supernatant was aspirated, and the platelets were resuspended in 1 mL of PBS, 15% acid citrate dextrose, and 0.1% BSA, then the platelets were repelleted. The platelets were resuspended to their original volume with autologous platelet-poor plasma containing prostaglandin E₁ (100 nmol/L) and apyrase (0.1 U/mL). The samples were then treated with FITC–anti-abciximab reagent (40 μg/mL), fixed, and analyzed on the FACScan as described above. The flow cytometric calibrations were performed with the same lot of FITC–anti-abciximab that was used to analyze patient samples.

Statistical Analyses
Patient demographics and baseline platelet function data are expressed as means, medians, ranges, and ± 1 SD of the mean. For continuous measurements of platelet aggregation and flow cytometric measurements, individual data points and medians were displayed graphically. Linear regression and 95% CIs were applied to assess the relationship between the radiometric and flow cytometric GP IIb/IIIa receptor occupancy assay values (Figure 5).

Results

Patient Demographics and Baseline Platelet Function Data
The patient demographics and baseline platelet function parameters for the study population at the time of enrollment are outlined in Tables 1 and 2. There was an equivalent distribution of both light-weight (<70 kg) and heavier (≥70 kg and <80 kg) individuals in the weight-adjusted and non-weight-adjusted treatment groups. However, the median and average weights in the non-weight-adjusted group were higher because an additional 8 subjects weighing ≥80 kg were enrolled in this arm. These subjects were included in this treatment group because all patients weighing ≥80 kg in the EPILOG trial received the 10-μg/min infusion. The groups were also well matched with regard to age and sex. Of the 41 subjects, 24 (58%) had a history of cardiovascular disease, and the majority of these subjects (17) were in the non-weight-adjusted infusion group. The median baseline platelet count for all subjects was 247 000 cells/μL, and the platelet counts of all subjects were within the normal range of 150 000 to 450 000 cells/μL, except for 1 patient who had a baseline platelet count of 129 000 cells/μL. Platelet counts were monitored for 28 days after abciximab administration, and the mean platelet count results are shown in Figure 1.

<table>
<thead>
<tr>
<th>Table 1. Patient Demographics</th>
<th>Total (n=41)</th>
<th>Weight-Adjusted Infusion (n=17)</th>
<th>Non-Weight-Adjusted Infusion (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>Mean±SD</td>
<td>62.9±10.0</td>
<td>61.5±10.5</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>65.0 (43–79)</td>
<td>65.0 (45–74)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>Mean±SD</td>
<td>72.0±15.1</td>
<td>65.8±11.5</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>72 (46–106)</td>
<td>69 (46–79)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Female</td>
<td>17 (42)</td>
<td>7 (41)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>24 (58)</td>
<td>10 (59)</td>
</tr>
<tr>
<td>History of cardiovascular disease, n (%)</td>
<td>24 (58)</td>
<td>7 (41)</td>
<td>17 (70)</td>
</tr>
<tr>
<td>Insulin-dependent diabetes, n (%)</td>
<td>1 (2)</td>
<td>0</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Baseline Platelet Function Data</th>
<th>Total (n=41)</th>
<th>Weight-Adjusted Infusion (n=17)</th>
<th>Non-Weight-Adjusted Infusion (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count, ×10⁻³</td>
<td>Mean±SD</td>
<td>247±59.3</td>
<td>249±61.7</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>247 (129–436)</td>
<td>234 (154–341)</td>
</tr>
<tr>
<td>% Light transmittance aggregation, 5 μmol/L ADP</td>
<td>Mean±SD</td>
<td>55±10.0</td>
<td>54±9.3</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>54 (32–76)</td>
<td>53 (40–71)</td>
</tr>
<tr>
<td>Abciximab receptors per platelet*</td>
<td>Mean±SD</td>
<td>101 400±27 000</td>
<td>95 400±16 700</td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>98 200 (65 000–147 000)</td>
<td>92 100 (73 000–136 000)</td>
</tr>
</tbody>
</table>

*Values were rounded to the nearest hundredths.
the entire treatment group, the mean platelet counts remained within 10% of baseline values. Therefore, abciximab administration did not significantly alter circulating platelet levels of the test subjects within the 28-day monitoring period.

The ranges of baseline abciximab receptor numbers were variable among the 41 individuals, ranging from 65,000 to 147,000 (median, 98,100), and agree with previous estimates.20,21 All patients’ platelets had a brisk aggregation response to ADP. The median increase of the baseline light transmittance value was 54% (range, 32% to 76%).

Platelet Pharmacodynamics

The effect of abciximab treatment on platelet function was assessed by measurement of the extent of GP IIb/IIIa receptor blockade and inhibition of ex vivo platelet aggregation response to the agonist ADP. The level of GP IIb/IIIa receptor blockade and ex vivo platelet aggregation responses for individuals receiving the non–weight-adjusted and weight-adjusted infusion regimens are shown in Figure 2. During abciximab administration, equivalent levels of GP IIb/IIIa receptor blockade were observed in both treatment groups. Maximum GP IIb/IIIa receptor blockade was seen at the earliest time point (0.5 hours after abciximab bolus; Figure 2a and 2c). At this time, the median GP IIb/IIIa receptor blockade for all patients was 93% (range, 83% to 96%). Also, in both treatment groups, the extent of GP IIb/IIIa receptor blockade decreased slightly during the infusion phase of treatment. However, at the end of infusion, GP IIb/IIIa receptor blockade was maintained at >80% in the majority of test subjects. Two individuals in the non–weight-adjusted infusion group and 4 subjects in the weight-adjusted infusion group had GP IIb/IIIa receptor blockade levels of <80% at the termination of transfusion.

Equivalent levels of inhibition of the ex vivo platelet aggregation responses to ADP were observed in both treatment groups (Figure 2b and 2d). Maximum suppression of platelet function was observed at 0.5 hours after abciximab bolus, where the median aggregation response was 2% of baseline (range, 0% to 32%). At this time, only 1 individual (non–weight-adjusted treatment group) had a platelet aggregation response of >20% of baseline. A trend toward increased platelet function during the 12-hour abciximab infusion period occurred, with a median platelet aggregation at the end of infusion of 14% of baseline (range, 0% to 54%). At the end of the infusion, 3 individuals in the non–weight-adjusted treatment group and 1 in the weight-adjusted treatment group had platelet aggregation responses >20% of baseline.

Partial recovery from both GP IIb/IIIa receptor blockade and inhibition of ADP-mediated platelet aggregation was observed 12 hours after cessation of abciximab therapy. At this time, median GP IIb/IIIa receptor blockade dropped to 68% (range, 52% to 85%). Only 1 individual in the non–weight-adjusted group had GP IIb/IIIa blockade measurements >80%. Also, the recovery of the platelet aggregation response (median, 38% of baseline; range, 0% to 88%) was comparable to the degree of GP IIb/IIIa blockade. One individual in the non–weight-adjusted group and 3 individuals in the weight-adjusted group sustained platelet aggregation levels of <20% of baseline at 12 hours after cessation of therapy. Therefore, partial recovery of blockade of both GP IIb/IIIa receptors and platelet aggregation was observed in the
Ex Vivo Flow Cytometric Analysis

The distribution of abciximab on the circulating platelet population was monitored over a 15-day period by flow cytometry, and the results are summarized in Table 3 and Figure 3. The data are expressed as the median fluorescence channel number and percentage of cells within the sample with no detectable abciximab on their surfaces. Abciximab-negative platelets were defined as having a relative fluorescence intensity ≤10, because 90% of the cells within the baseline samples (ie, preabciximab bolus) had relative fluorescence intensities below this range. The median fluorescence channel number provides a qualitative and quantitative assessment of both the surface density of abciximab and the rate of clearance of platelet-bound abciximab. The flow cytometry data reveal that abciximab was bound to circulating platelets in the majority of subjects up to 15 days after abciximab treatment. As shown in Table 3, peak fluorescence intensity was observed at 0.5 hour after abciximab bolus, indicating saturation of surface GP IIb/IIIa receptors by abciximab, and from 3 to 15 days after abciximab administration, the level of fluorescence intensity of the platelet population gradually decreased (Figure 3). The individual patient values reveal a high degree of heterogeneity in the median fluorescence intensity per platelet within the patient population. This intersubject variability could be influenced by a number of factors, including the basal number of abciximab receptors per platelet, the rate of platelet turnover, and circulating platelet levels. However, the median fluorescence intensity of the platelets of 34 of the 38 individuals with 15-day flow cytometric measurements was >10, indicating that, at 2 weeks after abciximab administration, detectable levels of the agent were present on circulating platelets from the majority of individuals tested.

To quantify the number of abciximab molecules per platelet from the corresponding median fluorescence intensity values at 8 and 15 days, the FITC–anti-abciximab probe was calibrated against the radiometric GP IIb/IIIa receptor occupancy assay. Fluorometric and radiometric binding isotherms of abciximab were generated on platelets from 3 normal human donors, and representative curves from 1 donor are shown in Figure 4. These curves encompass subsaturating concentrations of abciximab and therefore only the linear ranges of the binding isotherms (0 to 2.5 μg/mL). For the represented radiometric and fluorometric curves, linear regression analysis revealed a strong correlation between the amount of abciximab added per sample and either the number of 125I-labeled abciximab molecules bound per platelet (r² = .999) or the relative fluorescence intensity (r² = .987). Equivalent results were obtained with platelets from 2 additional donors.

To correlate the molecules of abciximab per platelet with the observed level of fluorescence intensity, the values from the radiometric (molecules of abciximab per platelet) and the flow cytometric (relative fluorescence intensity) assays for the 3 donors at each respective abciximab concentration were plotted against each other, and the results are shown in Figure 5. This derivation of the data was possible because the abciximab concentrations (the x values) in both the radiometric and fluorescence assays were identical. Linear regression

### Table 3. Abciximab Distribution on Circulating Platelets

<table>
<thead>
<tr>
<th>Initial Injection</th>
<th>Baseline</th>
<th>0.5 h</th>
<th>12 h</th>
<th>24 h</th>
<th>3 d</th>
<th>8 d</th>
<th>15 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak fluorescence channel</strong></td>
<td>Mean±SD</td>
<td>2±0.08</td>
<td>168±19.6</td>
<td>155±14.9</td>
<td>140±11.7</td>
<td>133±6.0</td>
<td>61±3.6</td>
</tr>
<tr>
<td>Median</td>
<td>2</td>
<td>120</td>
<td>135</td>
<td>141</td>
<td>132</td>
<td>61</td>
<td>28</td>
</tr>
<tr>
<td>n</td>
<td>37</td>
<td>37</td>
<td>38</td>
<td>37</td>
<td>41</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td><strong>% Abciximab-negative cells</strong></td>
<td>Mean±SD</td>
<td>92±1.0</td>
<td>4.8±0.8</td>
<td>5.5±1.8</td>
<td>3.4±0.8</td>
<td>1.3±0.2</td>
<td>3.5±1.0</td>
</tr>
<tr>
<td>Median</td>
<td>92</td>
<td>2.6</td>
<td>1.9</td>
<td>2.1</td>
<td>0.7</td>
<td>1.2</td>
<td>3.8</td>
</tr>
<tr>
<td>n</td>
<td>37</td>
<td>37</td>
<td>38</td>
<td>37</td>
<td>41</td>
<td>36</td>
<td>38</td>
</tr>
</tbody>
</table>

0.5-h and 12-h values indicate measurements acquired during abciximab treatment.
analysis revealed a strong correlation ($r^2 = .963$) between the number of abciximab molecules per platelet and the median fluorescence channel number. The equation of the line was calculated to be $y = (491)x + 79$, where $x$ is the median fluorescence channel number and $y$ is the number of abciximab molecules per platelet. On the basis of the above equation, the molecules of abciximab bound per platelet at 8 and 15 days after treatment were calculated for each patient, and the results are shown in Figure 6. At 15 days after treatment, measurable circulating levels of abciximab were detected in 34 of the 38 individuals tested. For the treatment group, the relative median fluorescence intensity at 8 and 15 days after abciximab treatment corresponded to 29,100 and 13,300 abciximab molecules per platelet. On the basis of a median baseline receptor number of 104,400 GP IIb/IIIa molecules per platelet (Table 1), these levels of receptor occupancy correspond to 29% and 13% GP IIb/IIIa receptor blockade, respectively. Fluorescence histograms of a representative patient (01017) revealed that abciximab was continuously redistributing between circulating platelets (Figure 7). The predose histogram illustrates that platelets possess low endogenous fluorescence intensity before abciximab treatment and also confirms the specificity of the FITC–anti-abciximab reagent. In contrast, the histograms at 0.5 hours after abciximab bolus displayed a unimodal pattern of highly fluorescent platelets, demonstrating that abciximab was uniformly bound to the entire platelet population (Figure 7b). Flow cytometry analysis at times when there was no free abciximab in the circulation (1, 3, 8, and 15 days after abciximab bolus; Figure 7c, 7d, 7e, and 7f, respectively) all exhibited a unimodal cell population that gradually diminished in relative fluorescence intensity, indicating that the level of abciximab molecules per platelet gradually decreased over time. It is also important to note that the platelet population remained unimodal throughout the 15-day monitoring period, and a separate population of non–abciximab-coated platelets was never detected. The persistence of a single fluorescent population throughout the 15 days and the progressive reduction in the level of fluorescence intensity over time suggests that abciximab must equilibrate onto new platelets entering the circulation. If abciximab did not dissociate from the GP IIb/IIIa receptors, a negative abciximab-staining platelet population would appear over time as new platelets enter the circulation. In addition, the relative fluorescence intensity of the abciximab-staining platelet peak would not decrease, because the surface density of abciximab would remain
Discussion

The EPIC and EPILOG trials established that a 0.25-mg/kg bolus of abciximab led to a marked reduction in clinically significant cardiac events for 6 months after percutaneous coronary intervention in both high- and low-risk patients. In contrast, a bolus dose of abciximab was insufficient to protect against ischemic events. In contrast, data presented here and elsewhere establish that the EPIC and EPILOG infusion regimens of abciximab maintained potent blockade of GP IIb/IIIa receptors and platelet aggregation throughout the duration of abciximab treatment. From these observations, one could conclude that long-term prevention of ischemic events associated with coronary intervention requires an abciximab therapy regimen sufficient to sustain suppression of platelet function for more than 12 hours.

The pharmacodynamic profile of abciximab described in the present study confirms earlier reports that the bolus dose of abciximab is capable of saturating the circulating GP IIb/IIIa receptor pool. This is illustrated by the fact that blockade of both GP IIb/IIIa receptors and platelet aggregation is rapidly attained after initiation of therapy (this report and Reference 21). Because of the high affinity and rapid on-rate of the molecule for the GP IIb/IIIa receptor, 80% GP IIb/IIIa receptor blockade is achieved in most individuals by administration of a bolus dose that provides less than the twofold excess of the total number of molecules needed to bind all GP IIb/IIIa receptors on circulating and splenic platelets. For the majority of treated individuals, the bolus dose constitutes the preponderance of the entire abciximab dose. As an example, the bolus represents ~75% of the total dose administered to an 80-kg individual. Abciximab also possesses a slow, yet appreciable dissociation rate from platelets, which explains why only a small fraction of additional abciximab is required to maintain high-grade platelet inhibition during the subsequent 12-hour infusion.

The weight-adjusted and non-weight-adjusted infusion regimens sustained equivalent levels of GP IIb/IIIa receptor blockade throughout the course of abciximab treatment in both light and heavy test subjects. However, a number of caveats should be noted before these pharmacodynamic profiles are extrapolated to the clinical situation. Most important, the present study was not performed either on the patient population or under the clinical conditions in which abciximab is prescribed. Therefore, the impact of heparin and other commonly prescribed cardiovascular drugs and the absence of arterial injury and acute coronary syndromes on the pharmacodynamics of abciximab were not evaluated. Several lines of evidence indicate that the potential for platelet activation is greater for patients undergoing percutaneous coronary interventions than for the normal population. Therapeutic levels of heparin have been shown to enhance platelet activation both in vitro and in vivo. The denudation of the arterial lining during angioplasty creates a prothrombotic surface onto which platelets adhere and aggregate. The interaction of platelets with the subendothelial surface could trigger the externalization of internal GP IIb/IIIa pools, thereby increasing the total number of GP IIb/IIIa receptors needed to be blocked for abciximab to have therapeutic benefit. Because the platelet activation state of patients undergoing percutaneous coronary interventions is probably greater than in our study population, higher levels of abciximab may be required in percutaneous coronary angio-
plasty patients to effectively depress platelet function. However, the platelet-inhibitory profile of abciximab exhibited in the subjects reported here mirrored other extended pharmacodynamic studies performed on smaller groups of patients undergoing percutaneous coronary angioplasty.16–18,21 Also, the dramatic reduction in ischemic events observed in the EPILOG trial indicates that the weight-adjusted infusion was sufficient to sustain long-term therapeutic benefit.2

Partial restoration of platelet function and a decrease in GP IIb/IIIa receptor blockade <80% were observed in the majority of patients within 12 hours after abciximab treatment. These data are in agreement with other extended pharmacodynamic studies16–18,21 that have shown that after termination of the abciximab infusion, the return of platelet function is tapered. In previously reported studies, partial inhibition of platelet function was maintained for 12 hours after the infusion, whereas platelet function was restored to normal ranges within 24 to 36 hours after treatment.16,21 The present study, as well as previously reported ex vivo and flow cytometric analyses,26 indicates that the gradual recovery from inhibition of aggregation was due to a tapered decrease in abciximab-mediated GP IIb/IIIa blockade among the entire platelet population and not to an averaging effect of untreated platelets entering the circulation. To obtain a quantitative assessment of abciximab binding to platelets at extended periods, the present study used flow cytometric measurements that were calibrated against the radiometric GP IIb/IIIa receptor blockade assay. These quantitative estimations revealed that substantial levels of abciximab remained on circulating platelets 2 weeks after treatment. At 8 and 15 days after treatment, the median numbers of abciximab molecules per platelet were 29 100 and 13 300, which corresponded to GP IIb/IIIa receptor blockade levels of 29% and 13%, respectively. This degree of GP IIb/IIIa receptor occupancy has little effect on platelet reactivity in response to typical agonists used to assess pharmacological function.29 However, low-level GP IIb/IIIa blockade exerts more subtle pharmacological effects, as demonstrated by the decrease in shear-induced large aggregate platelet formation 1 week after abciximab treatment.27

The persistence of a unimodal fluorescent platelet pattern throughout the 14-day monitoring period is interpreted to result from continuous in vivo redistribution of abciximab among circulating platelets. A second peak of platelets staining negatively for abciximab was not detected during this period. Also, the median fluorescence intensity of the cell peak gradually decreased over time, indicating that the surface density of abciximab on platelets was gradually diminishing. A pattern of gradually decreasing fluorescence is evidence that abciximab dissociates from the originally targeted platelet, which would otherwise retain the highly fluorescent profile that results from the initial saturation binding of abciximab. Other supportive evidence for continuous redistribution is that substantial levels of abciximab were present on circulating platelets longer than the normal platelet lifespan of 7 to 10 days.30 An alternative explanation for the extended presence of abciximab on all circulating platelets is the binding of abciximab to GP IIb/IIIa receptors on megakaryocytes, leading to new platelets being released into the circulation with abciximab on their surfaces. However, the fluorescence histogram patterns do not support this scenario. If abciximab remained permanently bound to the GP IIb/IIIa receptors throughout the life span of a platelet and new platelets entering the circulation contained abciximab on their surfaces, the fluorescence peak would remain monovariant, but the fluorescence intensity of this peak would not decrease over time, because the surface density of abciximab on these platelets would remain stable. Instead, the number of events corresponding to abciximab-coated platelets would gradually diminish as they were removed from the circulation.

The pharmacodynamic profile of abciximab confers the additional benefit of rapid reversibility of platelet inhibition by platelet transfusion. This principle was demonstrated in monkeys, in which transfused platelets rapidly restored and sustained hemostatic function in animals that received therapeutic levels of abciximab.31 The prompt recovery of platelet function was facilitated by both rapid clearance of unbound abciximab from the circulation18 and the redistribution of abciximab from platelets containing saturating levels of the drug to the transfused cells. More importantly, patients undergoing emergency coronary bypass surgery shortly after abciximab administration were reported to have acceptable mortality and bleeding complications.32 The persistence of abciximab in the circulation could potentially enhance its immunogenicity and/or immune system–mediated platelet clearance. In general, the incidence of an immune response for abciximab in the treated population is low.31 In the present study, the platelet counts of the study population remained stable throughout the period of 28 days after abciximab treatment, even though the majority of subjects had detectable circulating levels of abciximab 15 days after treatment.

In conclusion, the 0.25-mg/kg bolus and 12-hour, 10-mg/min infusion of abciximab elicits rapid, profound, and sustained inhibition of platelet function throughout the duration of treatment. In addition, the slow dissociation of abciximab from platelets results in a gradual recovery of platelet function that may contribute to the extended benefit in reduction of thrombotic complications after angioplasty.

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References


Pharmacodynamic Profile of Abciximab Therapy


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