Pharmacodynamics and Pharmacokinetics of Eptifibatide in Patients With Acute Coronary Syndromes
Prospective Analysis From PURSUIT

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Background—Platelet deposition and aggregation are central to the pathogenesis of ischemic complications of acute coronary syndromes (ACS). Pharmacodynamic effects of the platelet glycoprotein IIb/IIIa antagonist eptifibatide have been delineated in healthy subjects but not in patients with ACS. We assessed effects of eptifibatide on ex vivo platelet aggregation in patients enrolled in the Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin (eptifibatide) Therapy (PURSUIT) trial of ACS.

Methods and Results—Patients were randomly assigned to an intravenous bolus (180 μg/kg) and 72-hour infusion of eptifibatide (2.0 μg/kg per minute, n = 48) or placebo (n = 50). We assessed correlations of plasma eptifibatide levels with receptor occupancy and inhibition of ex vivo platelet aggregation at 5 minutes and 1, 4, 24, 48, and 72 hours during treatment and 4 and 8 hours after termination of infusion. Blood was collected in buffered citrate and D-phenylalanyl-L-prolyl-L-arginine chloromethylketone anticoagulants. Although eptifibatide produced profound, prolonged inhibition of platelet aggregation during therapy, aggregation appeared to recover partially by 4 hours after the bolus. The aggregation response was greater with thrombin receptor agonist peptide versus ADP stimulation; inhibition of platelet aggregation was greater in blood samples anticoagulated with citrate versus D-phenylalanyl-L-prolyl-L-arginine chloromethylketone (PPACK). Plasma eptifibatide levels correlated significantly with receptor occupancy but not with inhibition of platelet aggregation.

Conclusions—A bolus and infusion of eptifibatide inhibits platelet aggregation profoundly in patients with ACS and is followed by brief, partial recovery. These results enhance our understanding of the relation between pharmacodynamic and clinical effects of eptifibatide in such patients and may have important implications for its use in percutaneous interventions. (Circulation. 2001;104:399-405.)

Key Words: pharmacokinetics • platelets • coronary disease • glycoproteins

Platelet deposition and aggregation are integral to the pathogenesis of acute coronary syndromes (ACS) such as unstable angina and non–Q-wave myocardial infarction (MI).1 Agents that inhibit these processes, by antagonizing platelet glycoprotein (GP) IIb/IIIa, reduce MI and recurrent ischemia in patients with ACS.2–6 These agents block the binding of circulating fibrinogen and von Willebrand factor to GP IIb/IIIa, preventing the cross-linking of platelets necessary for aggregation and thrombosis.

Questions remain, however, about the relation between the pharmacodynamic and clinical effects of these agents. Measuring such an association is difficult because of both biological7–9 and procedural9–11 variables. For example, anticoagulants that chelate calcium, including buffered citrate and EDTA, reduce the affinity of GP IIb/IIIa for fibrinogen and may enhance its affinity for competitive receptor antagonists.9,11 When platelet aggregation is studied in blood collected with the use of these anticoagulants,
the inhibitory effect of the antagonist can be overestimated." Finally, although the platelet membrane contains an excess of GP IIb/IIIa receptors, the levels of receptor blockade and inhibition of platelet aggregation needed to prevent ischemic events in patients with ACS have not been established.

In experimental models of thrombolysis and reperfusion, blockade of ≈80% of platelet surface GP IIb/IIIa with a high-affinity antagonist has inhibited baseline ADP-induced platelet aggregation by ≈80%. This level may represent a threshold to prevent recurrent thrombosis. Patients treated with abciximab during MI or percutaneous coronary intervention (PCI) have shown similar levels of blockade, but rapidly reversible antagonists have not been studied in patients with ACS.

The Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrilin (eptifibatide) Therapy (PURSUIT) trial included 10,948 patients with ACS. Its substudy, PERIGEE (Precise Evaluation of Response to Integrilin [eptifibatide] Given for Elimination of cardiac Events), was designed prospectively to assess the pharmacokinetics of eptifibatide, inhibition of platelet aggregation, and receptor occupancy in this population.

**Methods**

**PURSUIT Protocol**

Patients were eligible for PURSUIT if they had had rest angina for ≥10 minutes within the preceding 24 hours, with ischemic ECG changes or elevated creatine kinase-MB. Patients enrolled in PERIGEE were randomly assigned to receive a 180-μg/kg bolus of eptifibatide with a 2.0–μg/kg per minute infusion for ≈72 hours or placebo. Infusions could continue for another 24 hours if PCI was performed. Patients received 325 mg aspirin daily and heparin adjusted to maintain an activated partial thromboplastin time of 55 to 70 seconds. Other than study drug treatment and blood sample collection, treatment was at the discretion of treating physicians.

**PERIGEE Study Design**

The 13 participating sites (list at http://dcri.mc.duke.edu/research/publications.html) evaluated consecutive patients meeting the PURSUIT criteria for inclusion in PERIGEE. All patients provided informed consent, and each institutional review board approved the protocol. Patients were excluded from PERIGEE if none of the specified analyses could be or were provided.

Blood samples were collected before bolus dosing; at 5 minutes and 1, 4, 24 (steady-state conditions), 48, and 72 hours of infusion; and at 4 and 8 hours after infusion. Samples at 48 and 72 hours were omitted if treatment ended before these points. Samples were collected in tubes containing d-phenylalanyl-l-prolyl-l-arginine chloromethylketone (PPACK), a tripeptide thrombin inhibitor that does not alter free plasma calcium concentration. At each time point we assessed (1) platelet aggregation, with a thrombin receptor agonist peptide (TRAP, SFLLRN) and ADP; (2) plasma eptifibatide levels; and (3) receptor occupancy. Blood for platelet aggregation and receptor occupancy testing also was collected in tubes containing 0.1 mol/L buffered citrate before infusion, at 24 hours of infusion, and at 4 and 8 hours after infusion. Personnel performing the analyses were unaware of treatment assignment and outcomes.

**Platelet Function Testing**

All sites received training to ensure standard performance of assays. Platelet aggregation was assessed with the technique of Born (see http://dcri.mc.duke.edu/research/publications.html). Raw values for platelet aggregation were the maximal amplitudes of aggregation after 4 minutes. All aggregometry tracings were evaluated at a core laboratory (University of Tennessee).

For receptor occupancy studies, samples were sent by overnight carrier (at room temperature) to the core laboratory. The proportion of total receptors occupied was quantified by means of the techniques of Jennings (see http://dcri.mc.duke.edu/research/publications.html).

**Pharmacokinetic Sampling**

Samples for drug levels were collected in 5-mL tubes containing EDTA. Within 20 minutes after collection, samples were centrifuged at 1500g for 15 minutes at room temperature. The plasma was transferred immediately to polypropylene tubes and stored at −20°C until shipped for analysis. Samples were sent by overnight carrier to the analytical laboratory (Phoenix International Life Sciences, Inc, St-Laurent, Quebec), packed in dry ice. Samples were analyzed by validated liquid chromatographic/mass spectrometric methods with oleandomycin as the internal standard (details at http://dcri.mc.duke.edu/research/publications.html). Of the 438 plasma samples received, 436 were successfully analyzed with this method.

**Statistical Analysis**

Continuous data for baseline characteristics, outcomes, receptor occupancy, normalized platelet aggregation, and plasma eptifibatide concentration are reported as medians with 25th and 75th percentiles. We calculated the percentage of patients at each interval with ≥50%, ≥80%, and ≥90% inhibition of aggregation and receptor occupancy.

The Wilcoxon 2-sample rank-sum test was used to compare duration of drug administration for eptifibatide versus placebo. The Wilcoxon signed-rank test was applied to the difference between TRAP- and ADP-induced platelet aggregation values (for patients with values for both at the time point) for several comparisons of TRAP versus ADP; the difference between PPACK and citrate platelet aggregation values (if patients had both at the time point) for several comparisons of PPACK versus citrate; and the difference between 1-hour and 5-minute values of ADP- and TRAP-induced platelet aggregation, receptor occupancy, and eptifibatide concentration (for patients with values at both times). The Spearman (rank order) correlation coefficient was used to test for associations between eptifibatide concentration and both percent inhibition of platelet aggregation and receptor occupancy among patients with data for all 3 variables for a given time point. All probability values are 2-tailed.

**Results**

**Patients**

Overall, 100 patients were enrolled in PERIGEE; 1 patient was enrolled but completed no assessments, and 1 was in a terminated eptifibatide arm. Data were available for the remaining 98 patients from the eptifibatide (n=48) and placebo (n=50) arms. PERIGEE patients did not differ substantially at baseline from other North American patients in PURSUIT (Table 1); eptifibatide-treated patients were similar to those given placebo (data not shown). Of note, 59% of PERIGEE patients had MI at enrollment. All randomized patients in this analysis received study drug, for a median 71.8 (50.2, 72.0) hours for patients assigned to eptifibatide and 59.1 (30.1, 72.0) hours for placebo-treated patients (P=0.09).

**Inhibition of Platelet Aggregation**

Median inhibition of ADP-induced platelet aggregation (Figure 1) exceeded 80% of the baseline value 5 minutes after eptifibatide administration. Transient recovery of platelet aggregation occurred during the first 4 hours. We assessed changes in antiplatelet activity and plasma eptifibatide levels.
at 1 hour versus 5 minutes of infusion. The difference was positive and significant for ADP- and TRAP-induced platelet aggregation (both $P<0.0001$) and negative and significant for receptor occupancy ($P<0.0001$) and eptifibatide concentration ($P<0.0001$). Between hours 4 and 24, however, median inhibition returned to that observed earlier and remained fairly constant for the duration of the infusion. Aggregation values returned to 52% of baseline 4 hours after termination of the eptifibatide infusion ($n=7$) and to 80% of baseline by 8 hours afterward ($n=6$).

Median inhibition of platelet aggregation was lower in response to TRAP than to ADP; this difference was present at

**Effect of Anticoagulant and Agonist on Platelet Aggregation**

Inhibition of platelet aggregation differed substantially between samples collected in PPACK versus citrate (Figure 2). Aggregation was inhibited more in citrate-anticoagulated samples at 24 hours than in PPACK-anticoagulated specimens, particularly for TRAP-induced aggregation. For eptifibatide-treated patients, both normalized and raw values of platelet aggregation for both ADP and TRAP were lower at 24 hours for citrate versus PPACK ($P<0.004$ for normalized/ADP; $P<0.0001$ for normalized/TRAP; $P<0.015$ for raw/ADP; and $P<0.0001$ for raw/TRAP).

Inhibition of ADP-induced aggregation was consistently less in PPACK-anticoagulated samples versus citrate at all time points with data available for both. Inhibition in PPACK-anticoagulated samples exceeded 80% in >80% of patients at all time points during infusion except 1 and 4 hours (proportions of 46% and 53%, respectively) (Table 2). Inhibition exceeded 90% in a minority of patients at all times except 24 and 48 hours. However, no time point did >20% of patients achieve ≥80% inhibition with TRAP agonist in PPACK-collected blood, although >80% of patients had ≥50% inhibition at 5 minutes, 24 hours, and 48 hours.

**Eptifibatide Concentration, Platelet Aggregation, and Receptor Occupancy**

Receptor occupancy (Table 3) and plasma eptifibatide levels followed very similar patterns and mirrored platelet aggregation results (Figure 3). Receptor occupancy and eptifibatide concentration fell during the immediate postbolus period, paralleling the transient increase in platelet aggregation, and
then recovered by hour 4. Receptor occupancy exceeded 80% immediately after the eptifibatide bolus in 45% of patients, and 65% of patients had ≥80% receptor occupancy at 24 and 48 hours.

When holding the time point constant, the relation between eptifibatide concentration and receptor occupancy appeared stronger than that between concentration and percent inhibition of platelet aggregation. Of the 8 Spearman rank-order correlation coefficients between concentration and percent inhibition (based on PPACK and ADP) at the 8 postbaseline time points, none was significant at the 0.05 level. For concentration and receptor occupancy (based on PPACK), however, 3 of the 8 values were significant (and positive): those at 5 minutes ($P=0.0028, n=32$), 24 hours ($P=0.0001, n=29$), and 48 hours ($P=0.0369, n=13$). At 24 hours into infusion, receptor occupancy correlated strongly with (log) eptifibatide concentration (Pearson $r=0.60; P=0.0006; Figure 4A$) but not with inhibition of ADP-induced platelet aggregation analyzed in PPACK ($r=0.09; Figure 4B$) or inhibition of TRAP-induced platelet aggregation ($r=0.02; Figure 4C$).

**Clinical Outcomes**

The primary clinical end point of death or MI at 30 days occurred in 5 patients in the eptifibatide group (10%) and in 9 placebo-treated patients (18%, Table 4). Major bleeding was slightly increased with placebo versus eptifibatide treatment.

**Discussion**

The current findings provide mechanistic support for previously observed clinical findings; these important observations may help guide further development of antiplatelet therapy. In PURSUIT, eptifibatide protected against ischemic events in patients with ACS; in the current study, eptifibatide provided immediate, sustained, and reversible inhibition of

### TABLE 2. Inhibition of ADP-Induced Platelet Aggregation: Eptifibatide-Treated Patients

<table>
<thead>
<tr>
<th>Percentage of Patients With Inhibition</th>
<th>≥50%</th>
<th>≥80%</th>
<th>≥90%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citrate PPACK</td>
<td>Citrate PPACK</td>
<td>Citrate PPACK</td>
</tr>
<tr>
<td>Baseline (n=37, 38)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>During infusion</td>
<td>5 min (n=37)*</td>
<td>100</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>1 h (n=26)*</td>
<td>96</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>4 h (n=15)*</td>
<td>100</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>24 h (n=33, 34)</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>48 h (n=17)*</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>72 h (n=9)*</td>
<td>100</td>
<td>89</td>
</tr>
<tr>
<td>Postinfusion</td>
<td>4 h (n=6, 7)</td>
<td>83</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>8 h (n=6, 6)</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>

*PPACK samples only.

### TABLE 3. Receptor Occupancy: Eptifibatide-Treated Patients

<table>
<thead>
<tr>
<th>Percentage of Patients With Occupancy</th>
<th>≥50%</th>
<th>≥80%</th>
<th>≥90%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citrate PPACK</td>
<td>Citrate PPACK</td>
<td>Citrate PPACK</td>
</tr>
<tr>
<td>Baseline (n=35, 37)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>During infusion</td>
<td>5 min (n=38)*</td>
<td>100</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>1 h (n=27)*</td>
<td>96</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4 h (n=14)*</td>
<td>100</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>24 h (n=32, 34)</td>
<td>97</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>48 h (n=18)*</td>
<td>100</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>72 h (n=9)*</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>Postinfusion</td>
<td>4 h (n=6, 6)</td>
<td>83</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>8 h (n=6, 6)</td>
<td>83</td>
<td>33</td>
</tr>
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</table>

*PPACK samples only.
platelet aggregation in a representative subset of these patients. The current report is the only study to compare simultaneous observations of platelet aggregation, occupancy of platelet GP IIb/IIIa, and plasma levels of a GP IIb/IIIa antagonist in a large cohort that is representative of a larger trial of clinical outcomes.

In highly thrombogenic models of coronary occlusion, profound inhibition of platelet aggregation has been required to prevent rethrombosis. Animal and ex vivo studies have indicated that methods previously used to select the dose of eptifibatide (and perhaps other GP IIb/IIIa antagonists) may have overestimated its platelet-inhibiting effect. These methods would have overrepresented the antiaggregatory effects of eptifibatide and may have led to underestimation of the dose required for clinical effectiveness.

Most studies of ex vivo platelet aggregation have used buffered citrate anticoagulation. Such anticoagulation reduces plasma concentrations of ionized calcium from 1.1 mmol/L to 40 to 50 μmol/L and can alter platelet aggregation. Phillips and colleagues have extended these in vitro observations to volunteer subjects given eptifibatide. They noted that the IC50 for eptifibatide varied by a factor of 4, depending on the ionized calcium concentration. They also reported a 7-fold difference in the IC50 of eptifibatide for labeled fibrinogen binding to platelets. The current study extends these modeled data to patients. The degree to which platelet aggregation was inhibited was significantly lower with PPACK versus citrate anticoagulation.

Within 1 hour after the start of the eptifibatide infusion in the current study, platelet aggregation was increased slightly compared with levels observed immediately after the bolus was given. This suggests a multicompartmental model of distribution for eptifibatide, with very high levels of inhibition of platelet aggregation present shortly after the bolus is given, followed by later redistribution of the compound.

TABLE 4. Clinical Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Eptifibatide (n=49)</th>
<th>Placebo (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death*</td>
<td>1 (2)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Myocardial infarction*</td>
<td>4 (8)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Recurrent ischemia*</td>
<td>6 (12)</td>
<td>8 (16)</td>
</tr>
<tr>
<td>Any stroke</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Death/infarction*</td>
<td>5 (10)</td>
<td>9 (18)</td>
</tr>
<tr>
<td>Death/stroke/reinfarction/revascularization</td>
<td>9 (18)</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Moderate/severe bleeding</td>
<td>5 (10)</td>
<td>11 (22)</td>
</tr>
</tbody>
</table>

Values are expressed as n (%). *At 30 days.
because PCI for ACS often is performed within this early time frame.22

In fact, this model was used to design a newer regimen of eptifibatide administration in which 2 bolus doses of 180 μg/kg were given 10 minutes apart. This regimen recently was shown to prevent ischemic complications after PCI.23 The current findings suggest that to ensure adequate inhibition of platelet aggregation, patients with ACS treated with a single bolus and infusion of eptifibatide should receive another bolus dose of eptifibatide if PCI is performed within a few hours after the first bolus is given.

In PERIGEE, platelet aggregation was more robust in response to TRAP versus ADP in both treatment groups. TRAP mimics thrombin-induced platelet stimulation by activating the cloned thrombin receptor (PAR-1) without causing fibrin formation by the soluble coagulation cascade.24,25 When thrombin stimulates platelets, internal GP IIb/IIIa stores are expressed on the platelet surface.26 TRAP may stimulate this same phenomenon.27 In equimolar concentrations, TRAP causes more platelet degranulation and surface GP IIb/IIIa expression than ADP, resulting in more receptors on the surface.28 Thus TRAP stimulation might be expected to increase the ability of platelets to aggregate and might confer resistance to GP IIb/IIIa antagonism.

Several authors have reported similar phenomena. In a report of abciximab-induced inhibition of platelet aggregation, inhibition after TRAP-induced aggregation was less than that observed in ADP-stimulated platelets but was attenuated significantly when exogenous abciximab was added.17 Lesser inhibition after stimulation with TRAP versus ADP also has been reported with the peptidomimetic GP IIb/IIIa antagonist lamifiban.10 Investigators in that study devised a correction to reflect that TRAP-induced aggregation was never totally inhibited.10 Because the ability of aggregometry to distinguish various levels of inhibition with ADP is limited when aggregation is inhibited by >90%,28 the intriguing possibility is raised that TRAP stimulation of ex vivo specimens may be useful clinically to distinguish effects among high doses of GP IIb/IIIa antagonists.

In this study, the correlation of plasma eptifibatide levels with GP IIb/IIIa receptor occupancy was much more robust than that of plasma levels with platelet aggregation. The degree of variation when determining plasma levels and receptor occupancy was lower than that for turbidimetric platelet aggregation for several reasons, including less dependence on such external factors as ambient temperature, lipid levels, and postprandial state. Because receptor occupancy is measured simultaneously with total GP IIb/IIIa density and D3 epitope expression, it has the advantage of not requiring reference to a baseline value that may be several days remote. At high levels of platelet inhibition, receptor occupancy may provide better quantification of antiplatelet activity than would turbidimetric aggregometry.

These results enhance our understanding of the pharmacodynamic correlates of the clinical benefit of GP IIb/IIIa antagonism in patients with ACS. This study provides a detailed pharmacodynamic characterization of a therapeutic regimen studied in a large cohort (n=9461). There was a prompt inhibitory response to the bolus, but aggregation later increased transiently as eptifibatide concentrations decreased. Particularly intriguing is that with a rapidly reversible antagonist of GP IIb/IIIa, receptor occupancy and aggregation response to TRAP rather than ADP may provide meaningful estimates of biological activity.

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References


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