

Tardiff BE, et al. Pharmacodynamics and Pharmacokinetics of Eptifibatide in Patients with Acute Coronary Syndromes: Prospective Analysis from the PURSUIT Trial. Appendices.

### **Platelet Function Testing**

Blood (10 mL) was obtained from a site remote to the drug infusion site. Samples were immediately placed into tubes containing either buffered citrate or PPACK. Platelet-rich plasma (PRP) was obtained by centrifuging the tube at 150 g for 15 minutes at room temperature. The supernatant (the PRP) was pipetted into a clean plastic tube, and the remaining blood recentrifuged for 15 minutes at 1500 g to obtain platelet-poor plasma (PPP). Platelet-rich plasma was adjusted to a platelet count of 250,000/ $\mu$ L by dilution with autologous PPP. Standard aggregometry techniques were used; the maximum change in light transmittance at 4 minutes was reported. Aggregometry was performed simultaneously with ADP (final concentration 20  $\mu$ mol/L) and TRAP (final concentration 5  $\mu$ mol/L) as the agonists.

Normalized platelet aggregation was calculated by dividing a patient's raw level of aggregation (the maximal amplitude deflection at 4 minutes) at a given time by the raw baseline level.

### **Receptor Occupancy Studies**

For receptor-occupancy studies, 1 mL of platelet-rich plasma, anticoagulated with citrate or PPACK, was transferred into polypropylene tubes at room temperature. We used the D3 binding Assay for Receptor Targeting (DART) method,<sup>1</sup> which measures receptor occupancy of platelet glycoprotein (GP) IIb/IIIa antagonists that induce the D3 ligand-induced binding site. The DART assay determines the proportion of overall GPIIb/IIIa receptors with bound drug, by measuring D3 binding on platelets obtained from time points after administration of drug compared with the total number of receptors measured by D3 after the *in vitro* addition of excess drug. Thus this assay does not require a predrug or baseline sample to evaluate the extent of blockade by drug. The DART assay is unaffected by antiplatelet drugs, and samples can be analyzed  $\leq$ 72 hours after blood collection.

<sup>1</sup>Jennings LK, White MM. Expression of ligand-induced binding sites on glycoprotein IIb/IIIa complexes and the effect of various inhibitors. Am Heart J 1998;135:S179-S183.

### **Plasma Eptifibatide Concentrations**

The analytical procedure involved the addition of IS and Type I water to 300  $\mu$ L of each plasma sample followed by solid-phase extraction (SPE) using SPEC C18 AR (SPEC 15 mg) Bond Elute cartridges. Each column was washed with Type I water and then eluted with methanol, and part of the resulting solution was injected into a liquid chromatograph equipped with a mass spectrometer. Quantitation was achieved by the peak-area-ratio method (eptifibatide vs. IS); concentrations of the calibration-curve standards, the quality-control samples, and the study samples were determined using linear regression (weighted 1/concentration). The adjusted lower limit of quantitation was established at 43.5 ng/mL. Calibration-curve standards and quality-control samples

met the criteria showing acceptable performance of the method during the analysis of the study samples.

**Participating PERIGEE Sites (number enrolled)**

Baylor College of Medicine/The Methodist Hospital, Houston (24); Duke University Medical Center, Durham (15); Mt. Sinai Medical Center, New York (13); Barnes Hospital/Washington University, St. Louis (15); The Cleveland Clinic Foundation, Cleveland (11); University of Ottawa, Ontario (8); Alamance Regional Medical Center, Burlington (4); UNC Hospital, Chapel Hill (4); Parkview Hospital, Ft. Wayne (3); Memphis VA Medical Center (1); University of Arkansas Medical Center, Little Rock (1); John L. McClellan Memorial Hospital, Little Rock (1).