Platelets play a fundamental role in the pathogenesis of acute coronary syndromes (ACS) and the development of ischemic events complicating percutaneous coronary intervention (PCI) (1,2). The development of inhibitors of platelet glycoprotein (GP) IIb/IIIa, which block the common final pathway leading to platelet aggregation, has extended the therapeutic options for reducing ischemic complications. To date, three GPIIb/IIIa inhibitors have been approved by the U.S. Food and Drug Administration: the chimeric (murine/human) monoclonal antibody (c7E3 Fab) abciximab (ReoPro), the nonpeptide tyrosine derivative tirofiban (Aggrastat) and the cyclic heptapeptide epifibatide (Integrilin). Six large placebo-controlled, double-blind trials using GPIIb/IIIa inhibitors in patients undergoing PCI have shown that all three agents reduce the rate of acute ischemic complications (3–8). However, only abciximab has been shown to improve long-term outcomes of PCI (9–11). Specifically, the significant reductions in death, in myocardial infarction (MI) and in the need for urgent revascularization conferred by abciximab were still present one to three years after the PCI (9–11).

Following the PRISM-PLUS and PURSUIT trials (12,13), there has been an increasing usage of tirofiban and epifibatide to treat ACS patients in the early phase of their clinical course. Some of these patients later require PCI and may benefit from switching to abciximab, for which long-term benefits have been reported. The question therefore arises: can abciximab be administered effectively and safely to patients who arrive at the catheterization lab treated with the short-acting inhibitors, tirofiban or epifibatide?

Small molecule GPIIb/IIIa inhibitors, such as tirofiban, can cause conformational changes within the GPIIb/IIIa complex and induce the formation of novel epitopes (14). These conformational changes may interfere with subsequent binding of abciximab to the GPIIb/IIIa receptor. However, in vitro studies have demonstrated that abciximab binding to platelets is not adversely affected by prior exposure to tirofiban (15). Moreover, abciximab has a substantially higher affinity for the GPIIb/IIIa receptor than tirofiban or epifibatide (16). Thus, we hypothesized that the presence of a small molecule GPIIb/IIIa inhibitor would not impede the inhibitory effect of abciximab on platelet function.

The aim of this study was to evaluate platelet function using various methods and to preliminarily assess the clinical safety of sequential treatment with tirofiban or epifibatide.
epitifibatide followed by abciximab with a short overlap in ACS patients undergoing PCI.

METHODS

Patients. Patients (n = 50) were eligible for the study if they had an ACS, defined as unstable angina at presentation, or an acute MI within the previous two weeks. All patients were scheduled to undergo PCI within 24 h of enrollment. Exclusion criteria included an acute MI within 48 h, history of hemorrhagic diathesis, any major surgery, gastrointestinal or genitourinary bleeding within six weeks, history of stroke within two years or a residual neurological deficit, concurrent administration of oral anticoagulants unless the prothrombin time was ≤1.2 times control, thrombocytopenia (<100,000 cells/μl), hemoglobin level <12 gm/dl, severe uncontrolled hypertension or use of a thrombolytic agent within 48 h. This study was approved by the institutional review board of the Mount Sinai Hospital, and informed consent was obtained from each patient. The study was performed from March to October 1999.

Study medications. The study was comprised of three patient groups. Group I consisted of 25 consecutive patients who received tirofiban followed by abciximab (tirofiban-abciximab group). Group II consisted of 10 consecutive patients who received epitifibatide followed by abciximab (epitifibatide-abciximab group). Group III consisted of 15 consecutive patients who served as controls and received only abciximab (abciximab control group). All patients received concomitant therapy with aspirin 325 mg orally daily, except for two who received clopidogrel because of aspirin allergy. All patients were given initial therapy with heparin—a bolus of 50 U/kg intravenously and an infusion of 7 U/kg—adjusted to achieve 1.3 to 1.5 times the control APTT value. Heparin was continued until about an hour before the PCI.

In the tirofiban-abciximab group, tirofiban (gift from Merck & Co. Inc. West Point, Pennsylvania) was administered with heparin as an intravenous bolus of 0.4 μg/kg/min for 30 min, followed by 0.1μg/kg/min infusion over 20 to 24 h (12). In the epitifibatide-abciximab group, epitifibatide (gift from COR Therapeutics, Inc., San Francisco, California) was administered with heparin as an intravenous bolus of 180 μg/kg (pushed), followed by 2.0 μg/kg/min infusion over 20 to 24 h (13). In the abciximab control group, no GPIIb/IIIa inhibitor was given with heparin before the patient's arrival at the catheterization lab.

Just before the PCI, after obtaining arterial access, abciximab was initiated in all patients with the tirofiban or epitifibatide infusions still maintained in the first two groups. Abciximab was administered at 0.25 mg/kg bolus intravenously (pushed), followed immediately by 0.125 μg/kg/min infusion administered continuously over 12 h. Tirofiban or epitifibatide were discontinued after 5 min of overlap with the abciximab infusion. After their discontinuation, heparin was administered at a bolus of 30 U/kg followed by additional boluses of 10 U/kg as necessary to maintain an activated clotting time ≥200 s. Heparin was not administered after PCI. Patients having stent deployment received clopidogrel 75 mg orally once daily for four weeks.

Blood samples. Blood was collected from the tirofiban-abciximab group and 10 patients of the abciximab control group in vacutainers containing 3.8% sodium citrate. The epitifibatide-abciximab patients and five patients of the abciximab control had blood collected in vacutainers containing D-Phe-Pro-Arg chloromethyl ketone dihydrochloride (PPACK) (17). In both combination therapy groups, six samples were taken: 1) at baseline (on aspirin and heparin therapy); 2) after the tirofiban or epitifibatide bolus; 3) on steady-state tirofiban or epitifibatide infusion, just before the abciximab bolus; 4) at the end of the 5-min overlap period, just before the discontinuation of tirofiban or epitifibatide; 5) at 4 h after the initiation of abciximab; and 6) at 10 to 12 h after the discontinuation of abciximab. The abciximab control patients had blood samples drawn at parallel times, with sample 4 taken 5 min after abciximab initiation. In all patients, samples 1, 2, 5 and 6 were drawn from the antecubital vein by venipuncture using a 21-gauge needle, and samples 3 and 4 were drawn from a 7Fr arterial access sheath.

Assessment of platelet function. PLATELET AGGREGATION. Turbidimetric platelet aggregation was performed in platelet rich plasma (PRP) in response to 20 μM ADP and 15 μM of thrombin receptor activating peptide (TRAP), as previously described (18). The extent of aggregation was defined as the maximal amount of light transmission reached within 6 min after addition of the agonist.

CONE AND PLATELET ANALYZER (CPA). The CPA test quantifies platelet deposition under flow conditions. In this test, anticoagulated whole blood is placed in polystyrene wells and circulated at high shear rate (1,300 s⁻¹) with a rotating cone (19). The wells are then washed with PBS, stained with May-Grünwald stain and analyzed with an inverted light microscope connected to an image analysis system (19). The results were expressed as the percentage of total surface covered by platelets.

FLOW CYTOMETRY ANALYSIS OF FIBRINOGEN BINDING. As previously described (20), platelet activation was quantified by flow cytometric analysis of fibrinogen binding. This assay
(Wak-Chemie Medical GMBH, Germany) incorporates FITC-conjugated chicken antifibrinogen antibody, PRP, HEPES buffer or HEPES-EDTA for the negative control and stimulation by 0.6 μM ADP. Samples were analyzed in a FACSCalibur (Becton-Dickenson) and were gated on log-forward versus log-side scatter to identify the platelet population. Platelets with fluorescence greater than a marker set according to the negative controls were identified as positive events. Results of platelet function in all three assays were expressed as percentage of baseline.

**GPllb/IIIa receptor binding assay.** This method was applied to four patients in each of the three groups. The method uses a flow cytometric assay to quantify binding of two monoclonal antibodies LYP18 (Mab1) and 4F8 (Mab2) to GPllb/IIIa receptors (21). Binding of Mab1 to GPllb/IIIa receptors has been shown to be inhibited by abciximab, whereas Mab2 binding is unaffected by it. In contrast, small molecular weight antagonists inhibit Mab2, but not Mab1, binding (21). We used the platelet GPllb/IIIa occupancy kit (Biocytex, France) to determine Mab1 and Mab2 binding in our study groups. This kit employs whole blood diluted 1:4 in PBS BSA buffer, a negative isotypic control, Mab1 reagent, Mab2 reagent, a calibrated bead suspension and a polyclonal antibody antimouse IgG–FITC. Samples were analyzed in a FACSCalibur (Becton-Dickenson) and gated on log-forward versus log-side scatter to identify the platelet or beads populations. A calibration curve was plotted using the mean fluorescence intensity of the beads and their corresponding number of monoclonal antibody molecules. Results were expressed as the total number of binding sites for Mab1 or Mab2 per platelet.

**Assessment of safety.** Complete blood count was taken at all six time points and before the patient’s discharge. Bleeding was defined according to the criteria used by the Thrombolysis in Myocardial Infarction (TIMI) trial (22). Severe thrombocytopenia was defined as a nadir platelet count below 50,000/μl. Mild thrombocytopenia was defined as a platelet count below 100,000/μl or a nadir below 50% of the baseline value.

**Statistical analysis.** Patient demographics and platelet function data were described using mean ± SEM. Platelet function results were analyzed using a two-way analysis of variance with repeated measures between the different groups (between-subject factor) and time points (within-subject factor). Analysis of variance was performed separately for each combination therapy group and its corresponding abciximab control group. Categorical variables were compared using the Fisher exact test. Analyses were performed using SPSS version 10 statistical software, and statistical significance was considered as p < 0.05.

**RESULTS**

**Patient demographics.** A total of 50 patients representing a typical population undergoing PCI were treated. Their baseline characteristics are outlined in Table 1. There were no significant differences in any of the parameters between the three groups. All coronary interventions were successful (<50% residual stenosis). Forty-six patients (92%) had stents placed in the target vessel, and four patients (8%) underwent balloon angioplasty.

**Safety outcomes.** There were no major bleeding episodes in any of the groups. Two patients in the tirofiban-abciximab group and one each in the eptifibatide-abciximab and abciximab control groups had minor bleeding (p = NS). No blood transfusions were required. In addition, five patients in the tirofiban-abciximab group and one each in the eptifibatide-abciximab and the abciximab control groups had episodes of mild mucocutaneous bleeding, which did not qualify as minor bleeding. There were no episodes of severe thrombocytopenia. One patient in the tirofiban-abciximab group, none in the eptifibatide-abciximab group and two in the abciximab control group had mild thrombocytopenia.

**Tirofiban-abciximab platelet function assays.** The 25 patients in the tirofiban-abciximab group were compared with 10 patients from the abciximab control group using citrate as the anticoagulant. Overall analysis of variance was significant for the time effect (p < 0.0001) and interaction term (p < 0.0001) for all platelet function assays used. The residual platelet aggregation in response to 20 μM ADP is shown in Figure 1. Platelet aggregation decreased from baseline (100%) after the tirofiban bolus, was not significantly changed during the tirofiban infusion, and decreased

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<th>Table 1. Patient Demographics</th>
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Data presented as mean ± SEM or number (percentage) of patients.
further after the abciximab bolus (decrease from 12.4 ± 3.4% during tirofiban maintenance to 2.6 ± 1% after abciximab [p < 0.003]). Platelet aggregation in response to 15 μM TRAP showed a similar trend but with higher residual values (aggregation of 35.9 ± 5.3% after the tirofiban bolus and a decrease from 33.4 ± 4.2% during tirofiban maintenance to 16.7 ± 2.3% after abciximab [p < 0.0001]).

The residual fibrinogen binding results in the tirofiban-abciximab and abciximab control groups are shown in Figure 1. The differences between the tirofiban bolus and maintenance points were nonsignificant. After the abciximab bolus, fibrinogen binding decreased significantly (from 25.6 ± 4.3% to 5.2 ± 1.9% [p < 0.0001]). Cone and plate(let) analyzer results showed a similar trend (platelet coverage of 19.3 ± 2.9% after the tirofiban bolus and a decrease from 23.6 ± 4.5% during tirofiban maintenance to 2.2 ± 0.8% after abciximab [p < 0.0001]). Cone and plate(let) analyzer photomicrographs of a representative patient are shown in Figure 2.

A comparison between the tirofiban-abciximab and abciximab control groups revealed that residual platelet function at the post-abciximab bolus point was always lower in the tirofiban-abciximab group; however, the difference was significant only for 20 μM ADP aggregation (p < 0.02) and not the other platelet assays.

Figure 1. Residual platelet function assessed by 20 μM ADP-induced aggregation (top) and flow cytometry analysis of fibrinogen–platelet binding (bottom) in 25 patients who received tirofiban followed by abciximab and in 10 patients who received only abciximab. Results are expressed as mean ± SEM percent of baseline aggregation. Administration of abciximab to tirofiban-treated patients caused a significant decrease in platelet function. Open square = tirofiban-abciximab group; solid circle = abciximab control group. Abx = abciximab; Maint = maintenance; Tir = tirofiban.

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Micrographs were taken at magnification ×10.

**Eptifibatide-abciximab platelet function assays.** The 10 patients in the eptifibatide-abciximab group were compared with five patients from the abciximab control group using PPACK as the anticoagulant. Overall analysis of variance was significant for the time effect ($p < 0.0001$) and interaction term ($p < 0.0005$) for all platelet function assays used. The residual platelet aggregation in response to 20 μM ADP is shown in Figure 3. Platelet aggregation decreased from baseline (100%) after the eptifibatide bolus and was not significantly changed during the eptifibatide infusion or after the abciximab bolus (2.8 ± 1.1% during eptifibatide maintenance and 1.2 ± 0.8 after the abciximab bolus [$p = \text{NS}$]). Platelet aggregation in response to 15 μM TRAP showed a similar trend but with higher residual values (aggregation of 17.5 ± 5.2% after the eptifibatide bolus and a decrease from 13 ± 4.5 during the eptifibatide maintenance to 6.1 ± 4.1% after the abciximab bolus [$p = \text{NS}$]).

The residual fibrinogen binding results in the eptifibatide-abciximab and abciximab control groups are shown in Figure 3. The differences between the eptifibatide bolus and maintenance points were nonsignificant. After the abciximab bolus, fibrinogen binding decreased significantly (from 17.2 ± 3% to 6.6 ± 3.1 [$p < 0.008$]). Cone and plate(let) analyzer results showed a similar trend (platelet coverage of 25 ± 3.8% after the eptifibatide bolus and a decrease from 21.3 ± 3.1% during eptifibatide maintenance to 1.9 ± 0.4% after abciximab [$p < 0.001$]).

A comparison between the eptifibatide-abciximab and abciximab control groups revealed that residual platelet function at the post-abciximab bolus point was always lower in the eptifibatide-abciximab group; however, the difference was not significant for any of the platelet function assays.

**GPIIb/IIIa receptor binding.** The mean number of Mab1 and Mab2 binding sites per platelet in the tirofiban-abciximab and eptifibatide-abciximab groups is displayed in Figure 4. After the tirofiban bolus and maintenance, the number of Mab2 sites decreased to 32.4% and 29.7% of the baseline value, respectively, whereas the number of Mab1 sites did not change significantly. The abciximab bolus caused an increase in Mab2 sites to 75.8% and a concurrent marked decrease in Mab1 to 9% of baseline. Eptifibatide had a similar effect. After the eptifibatide bolus and maintenance, the number of Mab2 binding sites decreased to 30.9% and 26.3% of baseline, respectively, whereas the number of Mab1 sites did not change significantly. The abciximab bolus again caused an increase in Mab2 sites to 99.6% and a concurrent marked decrease in Mab1 to 4.8% of baseline.

**DISCUSSION**

This is the first in vivo study of combination intravenous GPIIb/IIIa inhibitor therapy. Previously, only sequential parenteral-oral GPIIb/IIIa blockade therapy has been examined and was found to be synergistic in its pharmacodynamic effect (23). By assessing various aspects of platelet function—aggregation, fibrinogen binding and high shear rate-induced surface deposition—we demonstrated that effective and continuous platelet inhibition is achieved when full dose abciximab is administered to patients already receiving full dose tirofiban or eptifibatide. Platelet inhibition after the abciximab bolus, at the point when both drugs were given simultaneously, was consistently equal to or greater than that achieved by any of the three agents alone.

The study did not aim to compare the effects of the three GPIIb/IIIa inhibitors on platelet function. It was divided into two cohorts: tirofiban-abciximab and eptifibatide-abciximab, each with its own abciximab control group and anticoagulant. Citrate, as opposed to PPACK, has been shown to have a calcium-depleting effect with subsequent overestimation of platelet inhibition by eptifibatide (17). Thus, PPACK was used as the anticoagulant in the eptifibatide-abciximab cohort, while citrate was used for the tirofiban-abciximab cohort, to enable comparison to previous studies.

In all treatment groups, the highest residual platelet function values were generally achieved by platelet aggregation with 15 μM TRAP. These findings are consistent with previous reports of >60% residual 15 μM TRAP induced aggregation 2 h after an abciximab bolus (24). Stimulation of platelets with thrombin has been shown to cause up to a 50% increase in the number of GPIIb/IIIa receptors available on the platelet surface, through recruitment of recep-
tors from α-granules to the plasma membrane (25). This process may explain the increased platelet reactivity we have found in response to TRAP.

**Tirofiban-abciximab group.** The residual platelet function after tirofiban administration ranged from 12% to 26% when assessed by all platelet assays, except for 15 μM TRAP aggregation. These findings are consistent with a recent study that also assessed platelet function after tirofiban therapy (26). Administration of abciximab to the patients treated with tirofiban caused a further reduction in platelet function. Moreover, residual platelet aggregation after the abciximab bolus was lower than that achieved by abciximab alone in the abciximab control group. These findings are supported by previous in vitro data (15) and by the results of the GPIIb/IIIa receptor binding assay in our study. Both demonstrated that binding of abciximab to the GPIIb/IIIa receptor is not adversely affected by the presence of tirofiban.

**Eptifibatide-abciximab group.** Administration of abciximab after eptifibatide caused only minor nonsignificant declines in platelet aggregation. This is probably because aggregation was already markedly inhibited during the

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**Figure 3.** Residual platelet function assessed by 20 μM ADP induced aggregation (top) and flow cytometry analysis of fibrinogen-platelet binding (bottom) in 10 patients who received eptifibatide followed by abciximab and in five patients who received only abciximab. Results are expressed as mean ± SEM percent of baseline aggregation. A significant decrease in platelet function after the abciximab bolus was evidenced only by the fibrinogen-binding assay. **Open square** = eptifibatide-abciximab group; **solid circle** = abciximab control group. Abx = abciximab; Ept = eptifibatide; Maint = maintenance.
eptifibatide infusion (2.8% residual aggregation with 20 μM ADP), as previously reported (26). Therefore, only a limited further decline was achieved by the addition of abciximab. In contrast, both the fibrinogen binding and CPA assays showed significant decreases after the abciximab bolus. Our data, therefore, suggest that analysis of fibrinogen binding to activated platelets and quantification of platelet deposition under flow conditions may be more sensitive methods than aggregation for the detection of platelet function changes at high levels of GPIIb/IIIa receptor inhibition. This hypothesis is supported by previous findings that platelet aggregation is only minimally affected at receptor occupancy levels of <30% and >80% (27).

**GPIIb/IIIa receptor binding.** In both combination therapy groups, the abciximab bolus, administered when tirofiban or eptifibatide were still infusing, had a similar effect on binding of the monoclonal antibodies Mab1 and Mab2. As expected, the number of available Mab1 binding sites decreased markedly, indicating that prior treatment with tirofiban or eptifibatide does not interfere with binding of abciximab to the GPIIb/IIIa receptor. Surprisingly, at the same time, the number of available Mab2 sites increased.

![Figure 4. Binding of the monoclonal antibodies Mab1 and Mab2 to glycoprotein IIb/IIIa receptors on platelets.](image-url)
considerably, suggesting that the addition of abciximab may have resulted in displacement of tirofiban or eptifibatide from their binding site in the receptor. This displacement seems to be more complete with eptifibatide (increase in Mab^2 to 99.6% of baseline) than with tirofiban (increase to 75.8% of baseline). This is in accordance with the dissociation constants of abciximab, tirofiban and eptifibatide, which are 5, 15 and 120 nmol/L, respectively (16).

Safety outcomes. Of a total of 35 patients who received a small molecule GPIIb/IIIa inhibitor followed immediately by abciximab, three (8.6%) developed minor bleeding complications, and none developed major bleeding complications. This bleeding complication rate is similar to that of the abciximab control group (6.7%). It is also within the range of the major and minor bleeding complications observed in other GPIIb/IIIa studies (4–6,12,13). It should be noted that the CAPTURE, PRISM-PLUS and PURSUIT trials employed concomitant high-dose heparin (6,12,13), whereas heparin was given at a low dose both before and during PCI in this study. This difference could partly explain the relatively higher bleeding rates observed in the PRISM-PLUS and PURSUIT trials (12,13). The rate of thrombocytopenia (<100,000 platelets/μL) in our study was also relatively low—only one of the 35 patients who received combination therapy (2.9%).

Study limitations. The main limitation of this study was the relatively small sample sizes, especially for clinical safety assessment. In addition, patients were not randomly or blindly assigned to one of the three treatment groups although they were enrolled in a consecutive manner. Other limitations are the use of venous blood in samples 1, 2, 5 and 6 and arterial blood in samples 3 and 4 and the use of two different anticoagulants, which precluded comparison of the relative effects of tirofiban and eptifibatide on platelet function.

In conclusion, administration of abciximab immediately after tirofiban or eptifibatide effectively inhibits platelet function. Using concomitant low-dose heparin, this sequential GPIIb/IIIa regimen also appears to be safe although further clinical assessment is required. These findings may have important implications for patients with ACS treated with a short-acting GPIIb/IIIa inhibitor in the early phase of their clinical course. These patients may later require PCI and could benefit from treatment with abciximab, for which clear long-term benefits have been reported (9–11). Our short overlap strategy allows them to receive abciximab without a GPIIb/IIIa free interval, which may be hazardous in ACS.

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