PLATELET GpIIb/IIIa OCCUPANCY

Evaluation of anti GpIIb/IIIa platelet anti-aggregants by flow cytometry

Kit for 10 tests

(Patent US 6 168 925 / EP 0920628) **Ref. 7001**

For Research Use Only.
Not For Use in Diagnostic Procedures.

1 METHOD

Single color flow cytometric analysis of the GpIIb/IIIa glycoprotein receptor. The total number of platelet GpIIb/IIIa receptors and the number of free GpIIb/IIIa receptors (free of anti-aggregant molecules) are determined by converting the fluorescence intensity into corresponding number of sites per platelet based on a calibrated bead standard curve.

2 REAGENTS

- **Reagent 1:** 1 x 15 mL vial, diluent, 10-fold concentrated.
- **Reagent 2a:** 1 x 200 µL vial, negative isotypic control (mouse monoclonal antibody, IgG).
- **Reagent 2b:** 1 x 200 µL vial, MAb1 reagent, anti-GpIIIa (CD 61) monoclonal antibody.
- **Reagent 2c:** 1 x 200 µL vial, MAb2 reagent, anti-GpIIIa (CD 61) monoclonal antibody.
- **Reagent 3:** 1 x 400 µL vial, calibrated bead suspension. The beads are coated with increasing and accurately known quantities of mouse immunoglobulins IgG. The number of determinants coated on each bead population is indicated in the Assay Value Insert provided in the kit.
- **Reagent 4:** 1 x 800 µL vial, staining reagent, polyclonal antibody anti mouse IgG-FITC.

WARNING
All reagents contain sodium azide as a preservative.
Reagents containing sodium azide should be discarded with care to prevent the formation of explosive metallic azides. When dumping waste materials into sinks, use copious quantities of water to flush plumbing thoroughly

3 REAGENT PREPARATION AND STORAGE

Intact kits and contents are stable until the expiration date indicated on the box label, when stored at 2-8 °C.*

- **Reagent 1****
  Stability after opening: 2 months at 2-8°C, when free of contamination.
  Prepare a 1:10 dilution with distilled water. Prepare the appropriate volume required for the samples to be tested.
  Stability after dilution: 15 days at 2-8 °C.
- **Reagents 2a, 2b and 2c**
  Ready for use.
  Stability after opening: 2 months at 2-8 °C, when free of contamination.
- **Reagent 3**
  Ready for use.
  Shake vial well, 5 seconds, to resuspend beads before opening vial.
  Stability after opening: 2 months at 2-8 °C, when free of contamination.
- **Reagent 4**
  Ready for use.
  Stability after opening: 2 months at 2-8 °C, when free of contamination.

Notes: * Do not freeze the kit.
** The presence of crystals does not affect the quality of the reagent. Incubate at 37 °C until the crystals are completely dissolved.

4 SPECIMEN COLLECTION AND TREATMENT

- **Specimen collection**
  - Use non-wettable plastic blood collection tubes.
  - Blood is collected in 0.129 M / 0,109M trisodium citrate anticoagulant (using a ratio of 9:1 volumes).
- **Specimen preparation**
  - The test is performed either on citrated whole blood or on platelet rich plasma (PRP).
- **Specimen storage**
  When an anti-aggregant such as **blocking monoclonal antibody** is used (for example, 7E3 MAb) the blood specimen must be treated within 24 hours after collection.
  For any **other anti-aggregant** type, specimen storage time must be experimentally tested.
  Blood is stored at room temperature before testing (18-25°C).

5 PROCEDURE

Note: For good results exercise great care in the pipetting of small reagent volumes by depositing them at the bottom of the test tubes.

All reagents must be at room temperature.
One calibration curve must be run for each series. One series could contain up to 5 samples.

5.1. Reagent Tube SetUp

- Label 5 plastic tubes T1 to T5. Set the tubes in a rack.
- Pipette reagents into tubes as follows:
  Tube T1: Pipette **50 µL** of blood sample and add **150 µL** diluted Reagent 1. Mix.
  Tube T2: **20 µL** Reagent 2a (Negative control),
  Tube T3: **20 µL** Reagent 2b (MAb1),
  Tube T4: **20 µL** Reagent 2c (MAb2),
  Tube T5: **40 µL** Reagent 3 (shake vial well before pipetting).

5.2. Immuno-labeling of samples and control

- In each of tubes T2, T3 and T4 pipette **20 µL** diluted sample; vortex all tubes to mix.
- Incubate all tubes at room temperature for **20 minutes**.

5.3. Fluorescent Staining

- Pipette **20 µL** Reagent 4 in each of tubes T2 to T5; vortex all tubes to mix.
- Incubate all tubes at room temperature for **10 minutes**.
- Pipette **2 mL** diluted Reagent 1 into each of tubes T2 to T5.

Prepared samples may be stored for maximum **2 hours** at 2-8 °C before cytometric analysis.

5.4. Cytometric analysis

Refer to the Operator's Manual of the cytometer for instructions on how to perform cytometric readings.

The selected Mean Fluorescence Intensity (MFI) statistics is the geometric mean, Mn (x) or GeoMean.

Vortex each tube before analysis.

- Calibration analysis: tube T5 (Fig 1)

Create a FS LOG vs SS LOG cytogram. Add a discriminator to minimize the artefactual background. Set up a gate ("A") around the main bead population (Fig. 1a).

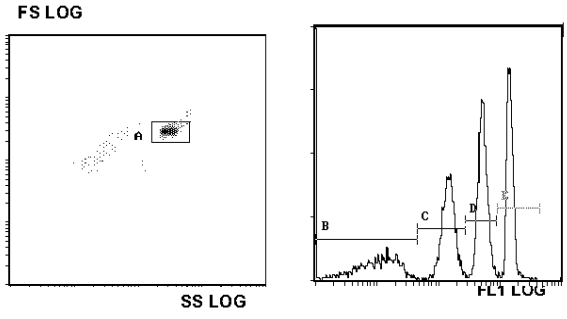
Create a FL_1 LOG gated by the "A" region.

Note the mean fluorescence intensity (MFI) for each of the 4 fluorescence peaks (Fig. 1b : B, C, D and E cursors) corresponding to the 4 calibration beads.

For optimum analysis conditions, the peak of the fourth bead fluorescence intensity (FL1) must be set at the beginning of the fourth decade. To achieve this, adjust the FL1 PMT voltage.

Fig. 1a :
Test calibration cytogram

Fig. 1b :
Cursor settings in gated fluorescence histogram



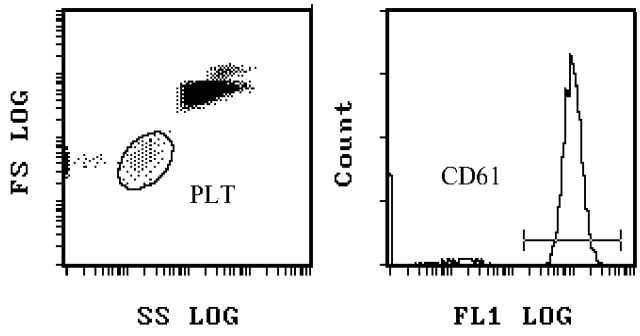
- Sample analysis (Fig 2)

Using the same acquisition procedure, on the FS LOG vs SS LOG histogram (Fig. 2a), platelets are isolated from other whole blood cells by an analysis region " PLT ".

In the corresponding gated fluorescence histogram (Fig. 2b), note the mean fluorescence intensity of each sample.

Fig. 2a :
Whole blood cytogram and platelet region gating

Fig. 2b :
CD61 (GpIIIa) immuno-labeling, cursor settings in PLT gated histogram



6 RESULTS

Depending on the instrument used:
If the MFI values (Mean Fluorescence Intensity) are expressed as linear values, use a log-log graph paper.
If the MFI values are obtained as channel numbers, use a semi-log graph paper.

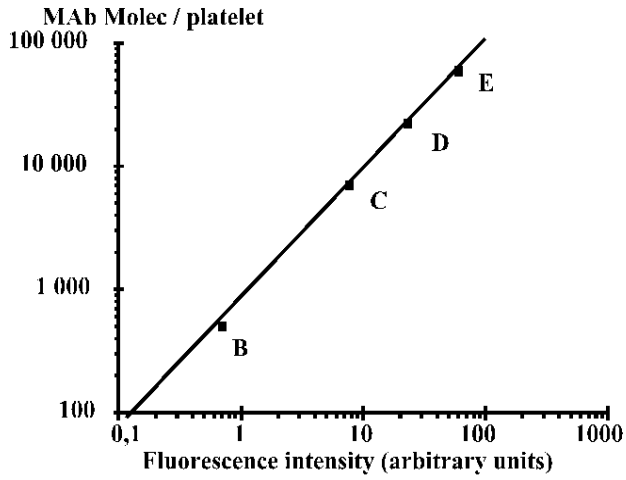
Using a log-log or a semi-log graph paper, plot the MFI calibration values on the abscissa (x-axis) and their corresponding number of monoclonal antibody molecules (as indicated in the assay value insert) on the ordinate (y-axis).

Draw the calibration curve.

Interpolate the MFI values of the tubes T2 to T4 (samples and control) on the calibration curve and read the corresponding numbers of monoclonal antibodies directly off the curve.

Specific quantitative MAb1 and MAb2 values are calculated after subtraction of the negative control measurement.

Example of calibration curve :



Expression of the results :

Antiaggregant type	Total number of GpIIIa sites (*)	Free GpIIIa sites (*)	Number of occupied receptors (*)	Occupancy ratio (%)
Monoclonal antibodies	MAb2	MAb1	MAb2 - MAb1	$\frac{(MAb2 - MAb1)}{MAb2} \times 100$
Peptides and peptido-mimetics	MAb1	MAb2	MAb1 - MAb2	$\frac{(MAb1 - MAb2)}{MAb1} \times 100$

(*) Values are expressed as numbers of MAb molecules bound per platelet.

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Quantification of abciximab-induced platelet inhibition is assay dependent: A comparative study in patients undergoing percutaneous coronary intervention

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Background The best method for measuring the degree of platelet inhibition with glycoprotein (GP) IIb-IIIa antagonists during percutaneous coronary intervention (PCI) and the optimal degree of periprocedural inhibition is uncertain. Low molecular weight heparins have been reported to cause less platelet activation than unfractionated heparin. Therefore, compared with unfractionated heparin (UHF), a low molecular weight heparin could enhance measured platelet inhibition. In this study, we compared 3 methods of measuring platelet inhibition and investigated the effects of half doses of abciximab in combination with either UFH or the low molecular weight heparin dalteparin in patients undergoing PCI with planned abciximab administration.

Methods Abciximab-induced platelet inhibition was measured serially by means of 3 assays: 1) GP IIb-IIIa receptor occupancy, 2) binding of the activated GP IIb—IIIa-specific monoclonal antibody PAC1, and 3) agglutination of platelets with fibrinogen-coated beads (RPFA). Forty patients were randomly allocated to receive either UFH (70 U/kg) or dalteparin (60 IU/kg), followed by a half dose of abciximab (0.125 mg/kg) administered twice at 10-minute intervals. Assays were obtained 10 minutes after each half dose of abciximab and 8 to 10 and 24 hours after abciximab administration.

Results No differences between UFH and dalteparin were observed. At each time-point measured, the mean percent platelet inhibition as determined by means of the receptor occupancy assay and PAC1 binding assay was less than the degree of inhibition determined by means of the RPFA.

Conclusions The results of targeted levels of platelet inhibition cannot be extrapolated between different clinical trials of GP IIb-IIIa antagonists unless the same assay is used. Dalteparin, compared with UFH, does not enhance platelet inhibition or receptor occupancy by abciximab, as demonstrated by means of 3 separate assays. (Am Heart J 2003;145: e6.)

Platelet glycoprotein (GP) IIb-IIIa antagonists have been demonstrated in controlled clinical trials to reduce adverse cardiac events in patients with acute coronary syndromes[1] and in patients undergoing percutaneous coronary intervention (PCI).[2,3] The results of the GOLD (AU-Assessing Ultegra) multicenter study[4] demonstrate that, in patients undergoing PCI, the degree of platelet inhibition as determined by means of the use of the Ultegra Rapid Platelet Function Analyzer ([RPFA] Accumetrics, San Diego, Calif) correlates with the development of major adverse cardiac events. In animal and clinical studies, ≥80% GP IIb-IIIa occupancy with abciximab is associated with reduced clinical events.[5] However, the interchangeability of different assays of GP IIb-IIIa antagonist-induced inhibition of platelet function remains uncertain.

Platelet activation results in an increase in the number of surface GP IIb-IIIa molecules.[6] Low molecular weight heparins (LMWHs) are associated with less platelet activation than unfractionated heparin (UFH).[7,8] Therefore, the use of LMWH may allow for reduced dosages of GP IIb-IIIa antagonist, while maintaining high levels of platelet inhibition similar to "full

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dose" GP IIb-IIIa antagonist therapy in conjunction with UFH.

There has been interest in measuring the degree of platelet inhibition by GP IIb-IIIa antagonist therapy. Although 80% inhibition is commonly held to be an appropriate therapeutic level, the exact level may depend on the type of assay used. In the recently published GOLD study,[4] a significant correlation was shown between platelet inhibition by a GP IIb-IIIa antagonist, as determined by means of the Ultegra RPFA and major adverse cardiac events (MACE). In this study, we therefore compared the Ultegra RPFA with 2 other typical methods for measuring platelet inhibition with GP IIb-IIIa antagonists: a GP IIb-IIIa receptor occupancy assay and binding of the activated GP IIb-IIIa-specific monoclonal antibody PAC1.

We addressed 2 issues in this study. First, in patients with unstable angina undergoing PCI with planned abciximab administration, we assessed the degree of platelet inhibition as measured by means of 3 independent assays. Second, we assessed whether the LMWH dalteparin, compared with UFH, enhances platelet inhibition with abciximab.

Methods

Percutaneous coronary intervention

After obtaining approval by the Institutional Review Committee of The Christ Hospital (Cincinnati, Ohio) and written informed consent of patients, we randomly assigned 40 patients with unstable angina pectoris (Braunwald class II-III[9]) and coronary anatomy suitable for PCI in a blinded fashion to receive either 60 IU/kg of dalteparin or 70 U/kg of UFH intravenously at the time of PCI. Five minutes after UFH or dalteparin administration, abciximab was administered intravenously in half of the standard bolus dose (0.125 mg/kg), followed in 10 minutes by the remainder (0.125 mg/kg). Initiation of the standard 12-hour intravenous infusion of abciximab (0.125 μg/kg/min, maximum 10 μg/h) began immediately after administration of the second abciximab bolus. Patients were excluded from enrollment when they had received any heparin during the 48 hours before PCI, had a known allergy to aspirin or thienopyridines, or had earlier abciximab-associated thrombocytopenia. Similarly, patients who had undergone recent (<6 weeks) surgery or trauma or had an earlier (within 6 months) stroke or gastrointestinal/genitourinary bleeding were excluded from participation. All patients received 325 mg of aspirin orally before and daily after PCI. Clopidogrel (300 mg load, 75 mg daily) was administered orally immediately after PCI in all patients who underwent coronary stent deployment. PCI was performed with standard techniques by experienced Ohio Heart Health Center operators (>300 PCIs/operator/y) at a single center (Christ Hospital). Vascular access sheaths were removed 4 hours after the study drug bolus was given. Hemostasis was maintained by means of local manual compression (30 minutes), followed by pneumocompression (Femostop, Radi Medical Systems, Reading, Mass) for 4 hours. Vascular closure devices were not used. Monitoring of anticoagulation (activated clotting time, activated partial thromboplastin time) was not performed, and no heparin was administered after PCI. The dose of UFH administered in this study (70 U/kg) was previously demonstrated to be safe and effective in randomized trials of abciximab therapy for PCI.[10,11] The dose of dalteparin (60 IU/kg) was selected from an earlier pilot trial experience, which demonstrated consistent antithrombotic efficacy (antifactor Xa activity exceeding 0.6 U/mL) and absence of clinical thrombotic events when administered in combination with abciximab in patients undergoing PCI.[12]

Assay time points and clinical outcomes

Platelet inhibition assays were performed as described on citrated (3.2%) venous blood obtained at baseline (before administration of either UFH or dalteparin), 5 minutes after administration of either UFH or dalteparin, 10 minutes after administration of each half-dose bolus of abciximab, 8 to 10 hours after administration of either UFH or dalteparin, and 16 to 24 hours after administration of either UFH or dalteparin. Clinical parameters assessed in the hospital included death, myocardial infarction (creatinine kinase-MB, or creatinine kinase in the absence of measured MB fraction, >3 times the upper limit of normal), and urgent revascularization (PCI or surgery). Bleeding events and requirements for blood transfusion were also assessed. Bleeding events were determined to be major or minor on the basis of the Thrombolysis In Myocardial Infarction (TIMI) Study Group designation, as previously described.[13]

Ultegra rapid platelet function assay

The Ultegra RPFA was used as previously described.[14] The RPFA is a means of measuring the increase in light transmittance of anticoagulated whole blood with time as a result of the agglutination of platelets (via unblocked surface GP IIb-IIIa) with fibrinogen-coated micro-beads, after platelet activation induced by means of iso-TRAP (thrombin receptor activating peptide).

Receptor occupancy assay

This whole blood quantitative no-wash assay of total and free GP IIb-IIIa receptors measured by flow cytometry has been previously described.[15] Citrated whole blood was mixed 1:1 (vol/vol) with a cocktail of kinase inhibitors (Beckman Coulter, Miami, Fla) (1/200 dilution final), and phosphatase inhibitors (Beckman Coulter) (1/50 dilution final), diluted in Hanks' Balanced Salt Solution (GIBCO BRL, Grand Island, NY), then placed at 4°C and shipped overnight to the Center for Platelet Function Studies at the University of Massachusetts Medical School in Worcester, Mass.

Our method was identical to that of Hezard et al,[15] except the samples were fixed in 0.5% formalin at the end of the labeling procedure. In brief, total GP IIb-IIIa receptors were measured by the binding of monoclonal antibody Mab2 (BioCytex, Marseilles, France), the binding of which to GP IIIa (CD61) is not inhibited by abciximab. Free GP IIb-IIIa receptors were measured by the binding of Mab1 (BioCytex) to GP IIIa, an interaction inhibited by abciximab. Analysis was performed in a FACSCalibur flow cytometer (Becton Dickinson, San Jose, Calif) equipped with a 488-nm argon ion laser, standard 3-color filter configuration, and CELLQuest cell analysis

software (Becton Dickinson). Calibration beads were used to determine the number of antibodies bound and to generate a standard curve to calculate the absolute numbers of occupied versus unoccupied GP IIb-IIIa receptors.

PAC1 binding assay

Monoclonal antibody PAC1 only binds to the activated conformation of GP IIb-IIIa and thus mimics the activation-dependent binding of fibrinogen to platelets.[16] Anticoagulated whole blood was first diluted 1:10 in modified HEPES Tyrode's buffer (137 mmol/L NaCl, 2.8 mmol KCl, 1 mmol/L $MgCl_2$, 0.4 mmol/L Na_2HPO_4, 0.35% bovine serum albumin, 5.5 mmol/L glucose), with a pH of 7.4, then added to buffer-containing saturating concentrations of fluorescein isothiocyanate (FITC)-conjugated PAC1 (Becton Dickinson) and the GP IIIa-specific monoclonal antibody RUU-PL7F12 conjugated to peridinin chlorophyll protein ([PerCP] Becton Dickinson) with 20 μmol/L iso-TRAP (Multiple Peptide Systems, San Diego, Calif). Samples were incubated at 22°C for 30 minutes, fixed with 0.5% formalin and HEPES (10 mmol/L), then placed at 4°C until shipment at 4°C to the Center for Platelet Function Studies at the University of Massachusetts Medical School.

Analysis was performed in a FACSCalibur flow cytometer. Platelets were identified by means of RUU-PL7F12-PerCP (GP IIIa) positivity and characteristic logarithmic forward and orthogonal light scatter. The threshold was set on FL3 to include only those events labeling positively for GP IIIa. Mean fluorescence intensity of PAC1 binding was determined on a single parameter histogram of PAC1-FITC (FL1) fluorescence displaying events from the GP IIIa-positive and platelet light scatter gates.

The percent platelet inhibition was calculated by use of the equation 100% minus the ratio of PAC1 geometric mean fluorescence intensity at any given time point relative to the baseline. PAC1 mean fluorescence intensity was stable for at least 96 hours in samples stored and shipped at 4°C (data not shown).

Statistical analysis

In comparing the distribution of baseline characteristics in randomized patients, differences in continuous variables were compared with *t* tests, whereas differences in categorical variables were compared by use of the χ^2 tests. All analyses were performed with SAS statistical software (SAS Inc, Cary, NC).

Results

Clinical characteristics and outcomes

Baseline characteristics of the 2 treatment groups are shown in Table I. There were no statistically significant differences between patients receiving UFH and patients receiving dalteparin in age, sex, history of myocardial infarction, unstable angina, hypertension, hypercholesterolemia, use of cigarettes, heart failure, peripheral vascular disease, obstructive lung disease, prior PCI or bypass grafting surgery, or medication use. Patients receiving dalteparin were more likely to have diabetes mellitus than patients receiving UFH.

Table I. Baseline characteristics by treatment group

Characteristic	UFH (n = 20)	Dalteparin (n = 20)	*P*
Age (mean)	61.5 ± 13.9	62.1 ± 10.7	NS
Female	6	6	NS
Medical history			
Prior myocardial infarction	4	4	NS
Unstable angina	10	13	NS
Hypertension	12	17	NS
Hypercholesterolemia	7	13	NS
Diabetes	3	10	0.016
Current smoker	6	4	NS
Prior smoker	8	7	NS
Heart failure	0	1	NS
Peripheral vascular disease	1	2	NS
Obstructive lung disease	2	1	NS
Prior PCI	9	4	NS
Prior bypass surgery	4	6	NS
Normal ECG	5	4	NS
Medications (pre-enrollment)			
β-Blockers	11	10	NS
Calcium-channel blockers	6	6	NS
Nitrates	6	4	NS
Diuretics	2	7	NS
ACE inhibitors	6	6	NS
Aspirin	19	18	NS

ACE, Angiotensin converting enzyme; *NS,* not significant; *PCI,* percutaneous coronary intervention; *UFH,* unfractionated heparin.

Sixteen patients in each group underwent stent placement. Four patients in each group had only balloon PCI. One patient in the UFH-treated group and 1 patient in the dalteparin-treated group had an elevation of creatine kinase-MB after the intervention. There were no bleeding complications in the patients randomized to receive UFH. Two patients receiving dalteparin had a minor bleeding event (hematuria and vascular access site oozing), and a hematoma developed in 1 patient in the dalteparin-treatment group. Thrombocytopenia developed in one patient receiving dalteparin.

Platelet inhibition

As shown in Table II, at each time point the mean percent platelet inhibition as determined by both the PAC1 assay and receptor occupancy assay was less than the inhibition determined by the RPFA.

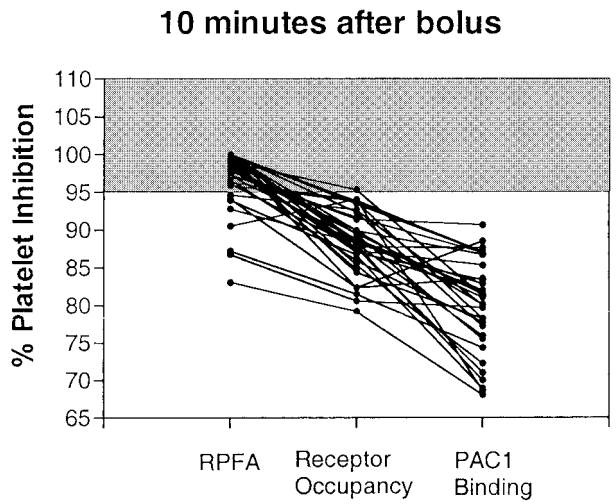
Ten minutes after the first half-dose bolus of abciximab was given, the mean percent platelet inhibition was 73% ± 3%, as determined by means of the RPFA (mean ± SEM), 57% ± 3% by means of the PAC1 assay, and 63% ± 3% by means of the receptor occupancy assay (Table II). At this time point, as determined by the Ultegra RPFA, 48% of patients achieved

Table II. Platelet inhibition as determined by 3 independent assays (n = 40 [LMWH and UFH] patients)

	1st 1/2 dose abciximab	2nd 1/2 dose abciximab	8-10 hrs	24 hrs
Mean percentage platelet inhibition ± SEM				
RO	63 ± 3	88 ± 1	81 ± 1	68 ± 1
PAC1	57 ± 3	80 ± 1	77 ± 3	75 ± 3
RPFA	73 ± 3	97 ± 1	92 ± 1	78 ± 1
Percentage of patients with ≥80% platelet inhibition				
RO	21	98	68	5
PAC1	8	61	51	55
RPFA	48	100	94	39
Percentage of patients with ≥95% platelet inhibition				
RO	0	3	3	0
PAC1	0	0	0	0
RPFA	9	79	44	0

PAC1, PAC1 binding assay; *RO*, receptor occupancy assay; *RPFA*, Ultegra Rapid Platelet Function Analyzer.

Figure 1



Percent platelet inhibition 10 minutes after the administration of the second bolus of abciximab, as determined by means of Ultegra RPFA, receptor occupancy assay, and the PAC1 binding assay in 40 patients with unstable angina undergoing PCI. The shaded area indicates patients with >95% platelet inhibition.

>80% platelet inhibition and 9% of patients achieved >95% platelet inhibition (Table II). As determined by the PAC1 assay, only 8% of patients achieved >80% platelet inhibition, and no patient achieved >95% platelet inhibition. As determined by the receptor occupancy assay, 21% of patients achieved >80% platelet inhibition, and no patient achieved >95% platelet inhibition (Table II).

Ten minutes after the second half-dose bolus of abciximab was given, the mean percent platelet inhibition was 97% ± 1% as determined by means of the RPFA, 80% ± 1% by means of the PAC1 assay, and 88% ± 1% by means of the receptor occupancy assay (Table II). At this time point, as determined by the RPFA, all patients achieved >80% platelet inhibition, and 79% of patients achieved >95% platelet inhibition (Table II). These data are similar to those reported in the GOLD study,[4] in which 10 minutes after the full bolus dose of abciximab was given the mean percent platelet inhibition was 96%, and 71% of patients achieved >95% platelet inhibition, as determined by the RPFA. In contrast to the RPFA, as determined by the PAC1 assay, 10 minutes after the second half-dose bolus of abciximab was given only 61% of patients achieved >80% platelet inhibition, and no patient achieved >95% platelet inhibition (Table II, Figure 1). Similarly, only 3% of patients achieved >95% platelet inhibition as determined by the receptor occupancy assay, whereas 79% of patients achieved >95% platelet inhibition as determined by the RPFA (Figure 1).

At the 8- to 10-hour time point, the mean percent platelet inhibition was 92% ± 2% as determined by the RPFA, 77% ± 3% as determined by the PAC1 assay, and 81% ± 1% as determined by the receptor occupancy assay (Table II), reflecting a slight decrease in the antiplatelet effects of abciximab despite the continuous intravenous infusion of this drug. Although 94% of patients achieved >80% platelet inhibition as determined by the RPFA, only 51% of patients achieved >80% platelet inhibition as determined by the PAC1 assay, and 68% of patients achieved >80% platelet inhibition as determined by the receptor occupancy assay (Table II). Consistent with these data, at the 8-hour time point in the GOLD study,[4] 94% of the subjects had >70% platelet inhibition as determined by the RPFA.

Table III. Platelet inhibition in patients randomized to receive dalteparin 60 IU/kg as determined by 3 independent assays (n = 20 patients)

	1st 1/2 dose abciximab	2nd 1/2 dose abciximab	8-10 hrs	24 hrs
Mean percentage platelet inhibition ± SEM				
RO	64 ± 4	87 ± 1	82 ± 1	67 ± 1
PAC1	55 ± 5	80 ± 1	73 ± 5	70 ± 6
RPFA	78 ± 3	97 ± 1	93 ± 2	78 ± 2
Percentage of patients with ≥80% platelet inhibition				
RO	15	100	65	0
PAC1	5	58	50	53
RPFA	50	100	94	50
Percentage of patients with ≥95% platelet inhibition				
RO	0	0	5	0
PAC1	0	0	0	0
RPFA	13	87	47	0

Table IV. Platelet inhibition in patients randomized to receive UFH 70 U/kg as determined by 3 independent assays (n = 20 patients)

	1st 1/2 dose abciximab	2nd 1/2 dose abciximab	8-10 hrs	24 hrs
Mean percentage platelet inhibition ± SEM				
RO	62 ± 5	89 ± 1	81 v 2	69 ± 2
PAC1	59 ± 5	80 ± 2	80 ± 2	79 ± 2
RPFA	69 ± 5	96 ± 2	92 ± 1	77 ± 1
Percentage of patients with ≥80% platelet inhibition				
RO	26	95	70	11
PAC1	11	63	53	58
RPFA	47	100	95	30
Percentage of patients with ≥95% platelet inhibition				
RO	0	5	0	0
PAC1	0	0	0	0
RPFA	5	74	42	0

At the 24-hour time point, the mean percent platelet inhibition declined to 68% ± 1%, 75% ± 3%, and 78% ± 1%, as determined by the receptor occupancy assay, the PAC1 assay, and the RPFA, respectively (Table II). Although 39% of patients still had >80% platelet inhibition as determined by the RPFA, 55% of patients had >80% platelet inhibition as determined by the PAC1 assay, and only 5% of patients had >80% platelet inhibition as determined by the receptor occupancy assay (Table II).

There were no significant differences between patients receiving dalteparin and patients receiving UFH (Tables III and IV).

Discussion

Inconsistent clinical benefits in trials of GP IIb-IIIa antagonists may reflect variable (and at times suboptimal) platelet inhibition.[1,17,18] In the recently published GOLD study,[4] 26% of patients had <95% platelet inhibition as determined by the Ultegra RPFA 10 minutes after intravenous bolus administration of a GP IIb-IIIa antagonist, and these patients experienced a significantly higher incidence (14.4% vs 6.4%, $P = .006$) of major adverse cardiac events ([MACEs] composite of death, myocardial infarction, and urgent target vessel revascularization). Furthermore, in the GOLD study,[4] patients whose platelet function was <70% inhibited, as determined by the RPFA, 8 hours after the start of therapy had a MACE rate of 25%, versus 8.1% for patients whose platelet function was >70% inhibited ($P = .009$). These data suggest the need for individualization of doses of GP IIb-IIIa antagonists.

As determined by the RPFA, this study showed a similar variation in platelet inhibition with abciximab to that reported in the GOLD study.[4] However, this study demonstrates that quantification of the degree of abciximab-induced platelet inhibition varies depending on the assay system used. For example, after the second half-dose bolus of abciximab was given, the percent of patients with >95% platelet inhibition was

79% with the RPFA, 3% with a receptor occupancy assay, and 0% with the PAC1 assay (Table II, Figure 1). It is therefore necessary for all platelet function assays to be rigorously tested in clinical trials of GP IIb-IIIa antagonists to determine the optimum degree of platelet inhibition for each device. Furthermore, this study demonstrates that the results of studies performed with different assays are not interchangeable.

Because LMWH activates platelets less than UFH does,[7,8] we hypothesized that the use of a LMWH such as dalteparin may allow reduced doses of GP IIb-IIIa inhibitors in these patients, because less GP IIb-IIIa molecules may be expressed on the platelet surface in an activation-dependent manner.[6] However, in this study of patients with unstable angina receiving abciximab, we demonstrated similar degrees of platelet inhibition with either UFH (70 U/kg) or dalteparin (60 IU/kg), as determined by the 3 independent assays. Thus, the modest platelet-activating effect of UFH does not appear to impact the dose of GP IIb-IIIa antagonist required to achieve therapeutic platelet inhibition.

Conclusions

The results of targeted levels of platelet inhibition cannot be extrapolated between different clinical trials of GP IIb-IIIa antagonists unless the same assay is used. Dalteparin, compared with UFH, does not enhance platelet inhibition or receptor occupancy by abciximab, as demonstrated by 3 independent assays.

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