Paradoxical inhibition of fibrinogen binding and potentiation of α-granule release by specific types of inhibitors of glycoprotein IIb–IIIa

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Abstract

Objective: To determine whether glycoprotein (GP) IIb–IIIa inhibitors can paradoxically augment activation of platelets, activation of GP IIb–IIia, α-granule degranulation, and lysosome release were induced after exposure of platelets to GP IIb–IIia inhibitors. Methods: ADP-induced platelet activation was assessed after exposure of platelets to Abciximab, or to a non-peptide ligand, the free acid of Orbostiban (Orbostiban). Activation of GP IIb–IIia was detected based on binding of fluorochrome labeled fibrinogen or a labeled monoclonal antibody, PAC-1. α-Granule degranulation was detected based on surface expression of P-selectin and lysosome release was detected based on surface expression of CD63. Results: Despite significant inter-individual variability in inhibition of fibrinogen binding in response to each of the GP IIb–IIia inhibitors used, a concentration dependent decrease in fibrinogen binding was seen with each agent in samples from each subject. Binding of PAC-1 was inhibited in a parallel manner. Abciximab increased ADP-induced P-selectin expression. Orbostiban did not alter ADP-induced P-selectin expression. Neither agent altered ADP-induced CD63 expression. When platelets were exposed to Abciximab and Orbostiban, both Abciximab and Orbostiban were found in the α-granules (by confocal microscopy), consistent with potentiation of agonist-induced release of α-granular products associated with uptake of proteins. Conclusions: Specific types of GP IIb–IIia inhibitors can paradoxically augment agonist-induced release of α-granules despite inhibiting agonist-induced fibrinogen binding. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Glycoprotein (GP) IIb–IIIa inhibitors inhibit aggregation of platelets by inhibiting binding of fibrinogen to the activated conformer of GP IIb–IIIa [1–3]. We have observed a phenomenon in which potentiation of agonist-induced α-granule release occurs in platelets that take up proteins such as fibrinogen [4]. Accordingly, this study was designed to determine whether the same thing occurs with inhibitors of GP IIb–IIIa and hence whether such agents can paradoxically augment ADP-induced α-granule degranulation reflected by the surface expression of P-selectin. Determination of the effects of antiplatelet agents on multiple components of activation is necessary because despite inhibition of aggregation the release of α-granular products by adherent platelets in vivo may exacerbate thrombosis by supplying coagulation factors V/Va and fibrinogen [5]. Furthermore, the release of growth factors (such as platelet derived growth factor and transforming growth factor β) present in α-granules may exacerbate atherogenesis [5].

Three types of GP IIb–IIIa inhibitors have been developed for intravenous use; (1) a chimeric Fab fraction of an antibody (Abciximab [ReoPro®]), (2) peptide ligands (e.g. epitifibatide [Integrilin®]), and (3) non-peptide ligands (e.g. tirofiban [Aggrastat®]). The administration of GP IIb–IIIa inhibitors in conjunction with coronary angioplasty is effective in reducing the incidence of cardiac events (myocardial infarction, death, and need for urgent revascularization) [6–9]. Treatment of patients with unstable angina or non-Q-wave myocardial infarction with GP IIb–IIIa inhibitors reduces the incidence of subsequent
cardiac events [10–12]. Adjunctive therapy with GP IIb–IIIa inhibitors in patients being treated with thrombolytic agents has increased early coronary patency [13,14]. The development of orally active preparations (such as Orbo®ban) should facilitate longer term treatment and may reduce further the incidence of cardiac events.

We have developed an assay in which the activation of platelets is induced in minimally altered whole blood by agonists such as adenosine diphosphate (ADP) and detected with the use of flow cytometry [4,15]. It entails the use of whole blood; provides sensitive and specific detection of selected components of platelet activation; utilizes physiologic concentrations of agonists; and permits analysis of the fixed platelets with flow cytometry long after sample acquisition. The assay permits assessment of diverse components of platelet activation: (1) Activation of GP IIb–IIIa can be identified by the binding of fibrinogen or an activation dependent antibody such as PAC-1; (2) α-granule degranulation can be identified by the surface expression of P-selectin; and (3) lysosome release can be identified by the surface expression of CD63.

In the present study, platelets were exposed to Abciximab, a chimeric Fab fragment, or to the free acid of Orbo®ban (Orbo®ban_a), a non-peptide inhibitor, in vitro. Platelet function in response to exposure to each agent was characterized by flow cytometry.

2. Methods

2.1. Patient population

In protocols approved by the University of Vermont Institutional Review Board, samples were obtained from healthy subjects who had not ingested aspirin or other non-steroidal anti-inflammatory agents for at least 10 days. After informed consent had been obtained, blood was obtained by peripheral venipuncture from all subjects. Three groups of subjects were recruited; (1) ten subjects whose blood was used for determination of the effect of Abciximab on fibrinogen binding and CD62 expression, (2) ten subjects whose blood was used for determination of the effect of Orbo®ban_a on fibrinogen binding and CD62 expression, and (3) five subjects whose blood was used for determination of the effect of Abciximab and Orbo®ban_a on PAC-1 binding and CD63 expression.

2.2. Collection of blood samples

Phlebotomy was performed with a two syringe technique in which the first 3 ml of blood were discarded. Blood to be analyzed with flow cytometry was drawn into syringes containing corn trypsin inhibitor (CTI, 32 μg/ml) alone and in combination with selected concentrations of Abciximab (0.01, 0.1, 1, 2, 4 and 10 μg/ml) or Orbo®ban_a (1, 10, 50, 100, and 250 ng/ml). CTI, a specific inhibitor of Factor XIIa without effect on other coagulation factors [16], was used as the anticoagulant because we have shown that the activation of platelets is altered by conventional anticoagulants such as citrate [15]. Platelets were exposed to GP IIb–IIIa inhibitors for 5–10 min before initiation of assays. To prevent clotting of blood during relatively prolonged exposure of the samples to Abciximab, blood was anticoagulated with 0.4 nM recombinant tick anticoagulant peptide (rTAP, kindly provided by Dr. George P. Vlasuk, Corvas International), 3 anti-IIa U/ml of recombinant hirudin (Sigma), or 1 anti-Xa U/ml of enoxaparin (Rhone–Poulenc Rorer) in addition to CTI.

2.3. Analysis of platelets by flow cytometry

Activation of platelets in response to adenosine diphosphate (ADP) was determined with respect to surface expression of P-selectin (α-granule degranulation), surface expression of CD63 (lysosome release), and with respect to binding of fibrinogen or PAC-1 (activation of surface GP IIb–IIIa). Surface expression of P-selectin was delineated with a phycoerythrin (PE) conjugated monoclonal antibody (anti-CD62, Becton Dickinson). Surface expression of CD63 was determined with a PE conjugated monoclonal antibody (anti-CD63, Immunotech, Marseille, France). Activation of GP IIb–IIIa was determined with fluorescein isothiocyanate (FITC) labeled PAC-1 (Becton Dickinson) or FITC–fibrinogen. Fibrinogen was labeled with celite FITC (Calbiochem, La Jolla, CA) as previously described [17]. Labeling of fibrinogen with celite FITC preserves the functional activity of fibrinogen and does not alter binding of fibrinogen to activated platelets [17]. Platelets were identified on the basis of size and the binding of a PerCP conjugated IgG directed against glycoprotein IIIa (anti-CD61, Becton Dickinson). Anti-CD61 binds to glycoprotein IIIa regardless of the activation state and does not interfere with binding of fibrinogen.

Assays were initiated by addition of 5 μl of whole blood to polypropylene tubes containing HEPES–Tyrode’s buffer (5 mM HEPES, 137 mM NaCl, 2.7 mM NaHCO₃, 0.36 mM NaH₂PO₄, 2 mM CaCl₂, 4 mM MgCl₂, and 5 mM dextrose, pH 7.4) and PerCP conjugated anti-CD61 (0.46 μg/ml) in addition to selected concentrations of ADP (0, 0.2, 1, 2 μM). Activation of platelets was determined with FITC conjugated fibrinogen (0.1 mg/ml) and PE conjugated anti-CD62 (1.15 μg/ml) or with FITC conjugated PAC-1 (2 μg/ml) and PE conjugated anti-CD63 (0.6 μg/ml). After 15 min platelets were fixed and red blood cells were lysed with Optilyse C (Immunotech). The association of antibodies with platelets was detected with the use of a fluorescence-activated cell sorter (Becton Dickinson). To quantify non-specific binding, control samples containing non-fractionated mouse IgG conjugated with PE or FITC and FITC conjugated albumin (for fibrinogen binding) were assayed for each subject. To confirm that an increase in the intensity of each activation
Fig. 1. ADP-induced P-selectin expression (left) and fibrinogen binding (right) in blood obtained from ten healthy subjects. Blood was anticoagulated with corn trypsin inhibitor (CTI, a specific inhibitor of factor XIIa) and exposed to 0, 1, 2, or 4 \( \mu \)g/ml of Abciximab (ReoPro) for 5–10 min before platelet assays were initiated. Surface expression of P-selectin (reflecting \( \alpha \)-granule degranulation) and fibrinogen binding were determined with the use of flow cytometry. Values are means±SEM. Fibrinogen binding in response to 0.2 and 1 \( \mu \)M ADP was inhibited \((P<0.001)\) by each concentration of ReoPro. Fibrinogen binding in response to 2 \( \mu \)M ADP was inhibited \((P<0.001)\) by 2 and 4 \( \mu \)g/ml of ReoPro. Increased P-selectin expression in response to 1 and 2 \( \mu \)M ADP was seen \((P<0.01)\) after exposure of blood to 2 and 4 \( \mu \)g/ml of ReoPro.

Depanulation

Fibrinogen Binding

dependent fluorochrome was caused by increased association of the specific fluorochrome with platelets, two additional controls were performed. In the first, blood was exposed to anti-CD61 PerCP to mark platelets, albumin–FITC, and anti-CD62 PE in addition to 2 \( \mu \)M ADP. In the second, blood was exposed to anti-CD61 PerCP to mark platelets, fibrinogen–FITC, and IgG PE in addition to 2 \( \mu \)M ADP. With respect to CD63, values reported are the percentage of platelets expressing CD63 in excess of binding seen in the absence of agonist.

2.4. Confocal laser scanning microscopy

The association with and localization in platelets of Abciximab and Orbo\( \text{fib} \text{a}\) \( \alpha \) were determined as previously described [4]. Abciximab was labeled with celite FITC, and blood anti-coagulated with CTI was exposed to 10 \( \mu \)g/ml of FITC–Abciximab or 250 ng/ml of Orbo\( \text{fib} \text{a}\) \( \alpha \) for 15 min. Platelets were fixed, and red blood cells were lysed with Optilyse C. The plasma membranes of the platelets were rendered permeable by addition of 0.1% Triton X-100 (Sigma, St. Louis). The Orbo\( \text{fib} \text{a}\) \( \alpha \) treated platelets were subsequently exposed to a mouse monoclonal anti-CD62 antibody for 15 min and then applied to a glass microscopic slide. The slide was washed, and an Alexa 568 conjugated goat anti-mouse IgG (Molecular Probes, Eugene, OR) was applied for 30 min. After additional washes had been performed on both sets, samples were air-dried, and a cover-slip was applied with 1% \( n \)-propyl gallate (Sigma) in 50% glycerol:50% PBS.

The slides were evaluated with the use of a BioRad (Hercules CA) MRC 1000 confocal scanning laser system equipped with a krypton/argon laser mounted on an Olympus BX50 microscope.

Platelets were imaged with the use of a 100× phase contrast oil immersion lens (N.A.=1.3) and an electronic zoom factor of 2.4. Phase contrast (non-confocal) images were acquired with the use of a transmitted light detector attachment. For platelets that were exposed to Abciximab, FITC–Abciximab was visualized with the use of 488 nm laser excitation, and P-selectin was visualized with 568 nm laser excitation. For Orbo\( \text{fib} \text{a}\) \( \alpha \)-treated platelets, the Alexa 568 conjugated secondary antibody localizing Orbo\( \text{fib} \text{a}\) \( \alpha \) was visualized with the use of 568 nm laser excitation, and FITC–anti-CD62 was visualized with 488 nm laser excitation.

Co-localization of Abciximab and P-selectin was delineated with the use of the multiply function in Macro.
Fig. 2. P-selectin expression and fibrinogen binding induced by 1 μM ADP in blood exposed to 10 μg/ml of Abciximab for up to 4 h. Blood was anticoagulated with CTI and 0.4 nM recombinant tick anticoagulant peptide. Increased ADP-induced P-selectin expression that was apparent from 10 min to 2 h after exposure to Abciximab was not seen after 4 h of exposure. Fibrinogen binding was inhibited throughout the entire interval. Results presented are from a representative experiment with blood from one healthy subject. Similar results were seen with blood from each of three subjects and when blood was anticoagulated with 3 anti-IIa U/ml of hirudin and 1 anti-Xa U/ml of enoxaparin.

Programming Language Software (BioRad). This function multiplies each pixel in the active display box by the corresponding pixel of a second image. After multiplication, the resulting image displays color only in areas where fluorescence was present in both images permitting colocalization of two fluorochromes.

Fig. 3. ADP-induced CD63 expression (left) and PAC-1 binding (right) in blood obtained from five healthy subjects. Blood was anticoagulated with corn trypsin inhibitor (CTI, a specific inhibitor of factor XIIa) and exposed to 0, 0.01, 0.1, 1, or 4 μg/ml of Abciximab (ReoPro) for 5–10 min before platelet assays were initiated. Surface expression of CD63 (reflecting lysosome release) and PAC-1 binding were determined with the use of flow cytometry. Values are means±SEM. PAC-1 binding in response to all concentrations of ADP was inhibited (P<0.001) by 1 and 4 μg/ml of ReoPro. CD63 expression was not altered by exposure of platelets to ReoPro.
2.5. Analysis of data

Values are means±SEM. Significance of differences was determined by analysis of variance. Differences between treatments were determined with the use of Student–Newman–Keuls tests. Significance was defined as *P*<0.05.

3. Results

3.1. Effects of Abciximab on platelet activation

Exposure of blood from ten subjects to 1, 2, and 4 μg/ml of Abciximab inhibited ADP-induced fibrinogen binding in a concentration dependent manner (Fig. 1). Each concentration of Abciximab inhibited (*P*<0.001) fibrinogen binding induced by 0.2, 1, and 2 μM ADP. By contrast, the ADP-induced P-selectin expression paradoxically increased after exposure of blood to Abciximab in a concentration dependent manner (Fig. 1). For example, P-selectin expression was increased (*P*<0.01) by exposure of blood to 2 and 4 μg/ml of Abciximab in combination with 1 and 2 μM ADP.

To determine whether exposure to Abciximab for up to 4 h resulted in a similar increase in ADP-induced P-selectin expression, blood was anticoagulated with rTAP in addition to CTI to prevent clotting of blood through 4 h. Increased P-selectin expression was seen from 10 min through 2 h after exposure to 10 μg/ml of Abciximab. However, it did not persist with 4 h of exposure (Fig. 2). Thus, the paradoxically induced degranulation appears to have a prompt onset and relatively prompt offset. Similar results were seen with blood that had been anticoagulated with recombinant hirudin and enoxaparin (data not shown).

To determine whether exposure to Abciximab potentiated the activation of GP IIb–IIIa, Abciximab (0.01, 0.1, 1, and 4 μg/ml) was added to blood from five subjects and activation of GP IIb–IIIa was detected with PAC-1. In addition, lysosome release was detected based on surface expression of CD63. Abciximab did not activate per se nor did it potentiate the activation of GP IIb–IIIa (Fig. 3). PAC-1 binding in response to 0.2 μM ADP decreased with each concentration of Abciximab (*P*<0.001). Abciximab did not alter ADP-induced release of lysosomes (Fig. 3). Inter-individual variability of inhibition of fibrinogen binding was apparent after exposure of blood to Abciximab (Fig. 4). Nevertheless, the inhibition was concentration dependent in each individual subject.

3.2. Effects of Orbofiban on platelet activation

The exposure of blood from ten healthy subjects to the active metabolite of Orbofiban, inhibited (*P*<0.001) ADP-induced binding of fibrinogen in a concentration dependent fashion (Fig. 5). Unlike results with Abciximab, exposure of blood to Orbofiban did not alter the threshold for ADP-induced α-granule degranulation (surface expression of P-selectin). Parallel results were seen with the free acid of Xemilofiban, another non-peptide ligand of the activated conformer of GP IIb–IIIa (data not shown).

To determine whether exposure to Orbofiban potentiated the activation of GP IIb–IIIa, Orbofiban (1, 10, and 100 ng/ml) was added to blood from five subjects. Activation of GP IIb–IIIa was detected with the use of PAC-1 and lysosome release was detected based on surface expression of CD63. Orbofiban did not activate per se nor did it potentiate the activation of GP IIb–IIIa (Fig. 6). A
Fig. 5. ADP-induced P-selectin expression (left) and fibrinogen binding (right) in blood obtained from ten healthy subjects. Blood was anticoagulated with corn trypsin inhibitor (CTI, a specific inhibitor of factor XIIa) and exposed to 0, 50, 100, or 250 ng/ml of the active metabolite of Orbofibran for 5–10 min before platelet assays were initiated. Surface expression of P-selectin (reflecting α-granule degranulation) and fibrinogen binding were determined with the use of flow cytometry. Values are means ± SEM. Fibrinogen binding in response to 0.2 and 1 μM ADP was inhibited \((P < 0.001)\) by each concentration of Orbofibran in all three groups. Fibrinogen binding in all three groups in response to 2 μM ADP was inhibited \((P < 0.001)\) by 100 and 250 ng/ml of Orbofibran. P-selectin expression was not affected by exposure of blood to Orbofibran under basal conditions or in response to ADP.

Fig. 6. ADP-induced CD63 expression (left) and PAC-1 binding (right) in blood obtained from five healthy subjects. Blood was anticoagulated with corn trypsin inhibitor (CTI, a specific inhibitor of factor XIIa) and exposed to 0, 1, 10, or 100 ng/ml of Orbofibran for 5–10 min before platelet assays were initiated. Surface expression of CD63 (reflecting lysosome release) and PAC-1 binding were determined with the use of flow cytometry. Values are means ± SEM. PAC-1 binding in response to all concentrations of ADP was inhibited \((P < 0.001)\) by 100 ng/ml of Orbofibran.
non-significant trend toward increased release of lysosomes was seen with 100 ng/ml of Orbofiban$_a$ (Fig. 6). Similar to the case with Abciximab, substantial inter-individual variation was seen in ADP-induced fibrinogen binding in response to a given concentration of Orbofiban$_a$. Nevertheless, a concentration dependent decrease in fibrinogen binding was seen in each individual subject (Fig. 7).

3.3. Uptake of Abciximab and of Orbofiban by platelets

We have previously shown that exposure of platelets to fibrinogen leads to its uptake into α-granules and an associated decrease in the threshold for surface expression of P-selectin (α-granule degranulation) induced by ADP or by the thrombin receptor agonist peptide (TRAP) [4]. Because one mechanism potentially responsible is an increased total mass of protein in α-granules, platelets were exposed to FITC–Abciximab and Orbofiban$_a$ before fixation and visualized with confocal laser scanning microscopy. Both Orbofiban$_a$ and Abciximab co-localized with P-selectin in α-granules of quiescent platelets (Fig. 8). Thus, if protein and peptide uptake stimulates α-granule release as implicated by results in previous studies [4,18], the exposure of platelets to a protein or a peptide inhibitor of GP IIb–IIIa may be a sufficient condition to induce the phenomenon.

4. Discussion

The development of GP IIb–IIIa inhibitors has improved the care of patients with acute coronary syndromes [6–14]. Characterization of the effects of these agents on selected components of platelet activation is likely to facilitate optimal implementation of therapy [19,20]. We used flow cytometry to determine whether exposure of platelets from healthy subjects to GP IIb–IIIa inhibitors altered α-granule degranulation and lysosome release in addition to blocking the binding of fibrinogen to the activated conformer of GP IIb–IIIa. As would be expected based on their mechanism of action, GP IIb–IIIa inhibitors did not inhibit activation of platelets but rather blocked binding of fibrinogen to previously activated conformers of GP IIb–IIIa.

We found that ADP-induced P-selectin expression was increased after exposure of blood to Abciximab but not to Orbofiban$_a$. P-selectin expression under basal conditions (no ADP) was low (<2% of platelets) and was not affected by exposure to Abciximab or Orbofiban$_a$. Further, we found that exposure of platelets to Abciximab and to Orbofiban$_a$ did not increase binding of the activation dependent antibody, PAC-1, to GP IIb–IIIa. Accordingly, neither Abciximab nor Orbofiban$_a$ activated platelets per se. By contrast, exposure of platelets to Abciximab augmented ADP-induced P-selectin expression. These results are consistent with those observed after exposure of platelets to fibrinogen [4]. We found that the exposure of platelets to increased concentrations of fibrinogen potentiated agonist-induced P-selectin expression associated with the uptake of fibrinogen into α-granules. Further, we have found that the exposure of platelets to a peptide inhibitor of GP IIb–IIIa, Eptifibatide, potentiated agonist-induced P-selectin expression [18].

We have postulated that an increased mass of protein in α-granules potentiates degranulation. The present results support this hypothesis. Exposure of platelets to Abciximab increased ADP-induced α-granule degranulation but did not alter ADP-induced lysosome release. Further, although both Orbofiban$_a$ and Abciximab co-localized in α-granules of quiescent platelets after exposure of platelets...
Fig. 8. The association of FITC–Abciximab (ReoPro) and Orbofiban with quiescent platelets in blood anticoagulated with CTI (32 μg/ml). Platelets were exposed to FITC–ReoPro (10 μg/ml) for 15 min (micrographs on left). The platelets were fixed in Optilyse C and exposed subsequently to 0.1% Triton X-100 and anti-P-selectin IgG. After washing, platelets were exposed to a secondary Alexa-conjugated anti-mouse IgG. The micrograph on the top (A) shows a representative platelet imaged with fluorescence (excitation 488 nm) demonstrating FITC–ReoPro (green signal). The micrograph in the center (C) depicts the same platelet visualized with fluorescence (excitation 568 nm) demonstrating P-selectin expression and localizing α-granules (red signal). The micrograph on the bottom (E) shows the same platelet viewed by multiplying fluorescent images (excitation 488 and 568 nm) and accordingly, the extent of co-localization of ReoPro–FITC and P-selectin (yellow color). The yellow color shows localization of ReoPro in α-granules. The micrographs on the right depict the association of Orbofiban with a quiescent platelet. Platelets were exposed to 250 ng/ml of Orbofiban for 15 min. Subsequently the platelets were fixed with Optilyse C and exposed to 0.1% Triton X-100 and anti-Orbofiban IgG. After washing, platelets were exposed to a secondary Alexa-conjugated anti-mouse IgG. P-selectin and thus α-granules were identified with FITC–anti-CD62. The micrograph on the top (B) shows a representative platelet imaged with fluorescence (excitation 568 nm) demonstrating Orbofiban (red signal). The micrograph in the center (D) depicts the same platelet visualized with fluorescence (excitation 488 nm) localizing α-granules (green signal). The micrograph on the bottom (F) shows the same platelet viewed by multiplying fluorescent images (excitation 488 and 568 nm) and accordingly, the extent of co-localization of Orbofiban and P-selectin (yellow color). Bar=10 μm, all images were obtained at the same magnification.

to the agents in whole blood, Orbofiban is not a protein and does not contribute to the overall mass of protein in α-granules. Accordingly, the lack of an effect of Orbofiban on ADP-induced P-selectin expression, despite its uptake into α-granules is consistent with the mechanism we have proposed. In addition, we have found that two other non-peptide ligands for GP IIb–IIIa, Xemilofiban and Tirofiban, do not potentiate ADP-induced P-selectin expression [18]. By contrast, Abciximab, an Fab fragment of IgG, and Eptifibatide, a peptide inhibitor of GP IIb–IIIa, potentiated agonist-induced α-granule degranulation [18]. These observations are consistent with the mechanism...
proposed because the uptake of both Abciximab and Eptifibatide contributes to the overall mass of protein in \( \alpha \)-granules.

The increased ADP-induced P-selectin expression seen after exposure of platelets to Abciximab was maximal during the first 2 h and was not apparent after 4 h of incubation of platelets in whole blood in vitro. This suggests that the potentiation of ADP-induced P-selectin expression may have a relatively rapid offset, consistent with recent observations in patients undergoing coronary angioplasty [21]. In addition, our results were obtained with blood from healthy subjects not taking aspirin or other medications. The studies reported here were designed to determine whether differential effects of selected types of GP IIb–IIIa inhibitors were present. Subsequent studies in blood from patients with coronary disease and in subjects ingesting other medications, particularly anti-platelet agents such as aspirin are required. In addition, in vivo studies with more prolonged exposure to selected agents should elucidate potential clinical implications.

A second possible explanation for the increased ADP-induced P-selectin expression seen with Abciximab is a partial agonist effect of Abciximab. Peter and colleagues found that Abciximab, particularly at low concentrations, can potentiate the activation of GP IIb–IIIa and hence the binding of fibrinogen and subsequent aggregation [22]. Thus, binding of ligands to GP IIb–IIIa may induce activation of platelets. This observation could explain, in part, the transient nature of potentiation of ADP-induced P-selectin expression seen in our studies. If the mechanism proposed by Peter et al. accounted for our results, the Abciximab that was initially bound to surface IIb–IIIa glycoproteins would, over time, be transported into \( \alpha \)-granules. Thus, any partial agonist effect would be attenuated by the trafficking of the surface bound ligand into the \( \alpha \)-granules.

Our results with respect to the potentiation of ADP-induced P-selectin expression by Abciximab are consistent with the mechanism proposed by Peter and colleagues [22]. By contrast, the lack of effect of Abciximab on ADP-induced CD63 expression (lysosome release) suggests that the potentiation of activation is not generalized but instead specific for \( \alpha \)-granule degranulation. Further, we did not see potentiation of binding of PAC-1 to GP IIb–IIIa even with low concentrations (0.01 and 0.1 \( \mu \)g/ml) of Abciximab. The use of assays in whole blood anticoagulated with CTI and the determination of activation of GP IIb–IIIa with PAC-1 may account for differences between our results and those of Peter and colleagues [22]. PAC-1 binds to the binding site for fibrinogen while Peter and colleagues used antibodies that bind to a ligand-induced binding site (LIBS). Anti-LIBS antibodies do not inhibit binding of fibrinogen to activated GP IIb–IIIa and thus recognize distinct epitopes [23]. Accordingly, our results are most consistent with a direct effect of the uptake of proteins into \( \alpha \)-granules on degranulation. Thus, they suggest that increased uptake of proteins into \( \alpha \)-granules potentiates agonist-induced degranulation.

Despite the efficacy of inhibition of fibrinogen binding demonstrated when data from each group of subjects were analyzed in aggregate, marked inter-individual variability was observed. However, a concentration dependent decrease in fibrinogen binding was observed in samples from each individual subject. Thus, our results suggest that after identification of a target level of inhibition of fibrinogen binding, titration of dose should be readily accomplished with the use of conventional pharmacokinetic principles.

Potent anti-platelet agents are potentially pivotal in improving the treatment of patients with coronary artery disease. Their availability has highlighted the need for improved assessment of diverse components of platelet function. The development of readily available, accurate, and targeted assays of platelet function should facilitate optimal titration of individual pharmacologic agents. In addition, such assays should permit enhanced characterization of diverse and potentially paradoxical effects of a given agent on platelet function that can facilitate its optimal clinical utilization.

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References


