Platelet Glycoprotein IIb/IIIa Inhibitors
Basic and Clinical Aspects

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Glycoprotein IIb/IIIa (GPIIb-IIIa) complexes (integrin αIIbβ3) mediate platelet aggregation by binding fibrinogen or von Willebrand factor (vWF), protein cofactors that form bridges between adjacent platelets. The cross-linked adhesive proteins assemble platelets into the aggregate. Agents that block the function of the GPIIb-IIIa complex of platelets constitute a powerful new generation of antithrombotic drugs. Among the short- and long-term aims of such drugs are (1) to provide immediate relief in the case of ongoing arterial thrombosis and (2) to eliminate excessive platelet reactivity in diseased vessels so that occlusive thrombi and restenosis do not occur, while allowing sufficient hemostasis to prevent spontaneous bleeding. It should be emphasized that stenosis and occlusion are both prothrombotic, with increased shear stress promoting platelet activation. Under these conditions, vWF plays a major role in the mediation of thrombus formation, interacting with GPIIb-IIIa and the adhesion receptor GP Ib. Otherwise, fibrinogen is the major cofactor of platelet aggregation, essentially binding through a dodecapeptide sequence (aa400 to aa411) present at the carboxy terminus of each γ chain. Binding of vWF and other adhesive proteins, such as fibronectin, to GPIIb-IIIa is mediated by the Arg-Gly-Asp (RGD) sequence, a universal mediator of cellular interactions with the extracellular matrix. Anti–GPIIb-IIIa drugs block this final step of the platelet aggregation process. They also block the “outside-in” signaling that follows the binding of adhesive proteins to activated GPIIb-IIIa and the onset of platelet aggregation. This signaling may promote events such as secretion, clot retraction, and the expression of procoagulant activity; therefore, its inhibition extends the influence of anti–GPIIb-IIIa drugs beyond the blocking of platelet-to-platelet cohesion.

GPIIb-IIIa Inhibitors Constitute a Wide Class of Drugs

The present review will mostly be illustrated by results obtained with abciximab (c7E3 Fab, ReoPro), a chimeric antibody fragment that is the most widely used of the new inhibitors. Abciximab acts rapidly; >80% of platelet receptors are blocked within 2 hours of the administration of a 0.25 mg/kg bolus in humans. Saturation is maintained during a 10-μg/min infusion (mostly between 12 and 24 hours), and recovery of platelet function is gradual after the infusion is stopped. Whereas plasma levels quickly fall, platelet-bound abciximab can be detected for up to 15 days after treatment. Flow cytometry shows that histograms representing bound drug within the total platelet population remain homogeneous while decreasing in intensity. The effect was defined in EPIC as a 35% relative risk reduction of death, myocardial infarction, or urgent revascularization within 30 days. An extended follow-up has since shown that abciximab also improves the probability of event-free survival over a long period. Although the risk of excessive bleeding was highlighted in the EPIC trial, weight-adjusted heparin dosing and early sheath removal in subsequent trials led to improved safety. The occasional need for emergency coronary bypass surgery is helped by the rapid clearance of abciximab from plasma, and platelet transfusion will provide functional platelets, lower the free plasma levels of the drug further, and promote the antibody exchange that has been suggested to occur between circulating platelets. Abciximab also substantially improves the safety of coronary stenting, now used in >60% of percutaneous revascularization procedures in the United States. Stenting can itself promote GPIIb-IIIa complex activation and predispose to coronary thrombus formation. The combination of aspirin and ticlopidine inhibits the thromboxane A2 and ADP-dependent pathways of platelet activation (Figure). However, multiple intracellular pathways appear to be involved in platelet activation, and these include serine and threonine phosphorylation of proteins by protein kinase C and phosphorylation of tyrosine residues on proteins by tyrosine kinase enzymes. However, different agonists use different pathways, and by blocking the end step of platelet aggregation common to all physiological agonists, anti–GPIIb-IIIa drugs provide a theoretically wider range of protection. GPIIb-IIIa inhibition is also now often considered as part of a last resort “rescue” therapy in the case of abrupt coronary occlusion or the failure of PTCA to restore the circulation. The concept is that this facilitates the dispersion of platelet-rich thrombi in difficult...
Schematic diagram showing the target for GPIIb-IIIa inhibitors. Platelet activation can be initiated by adhesion to immobilized substrates in the vessel wall or to a fibrin clot or by soluble agonists such as ADP and thrombin. Antiplatelet therapies can target individual receptor-linked activation pathways. For example, ticlopidine and clopidogrel act on an ADP-induced activation pathway. Aspirin dampens the platelet response by inactivating cyclooxygenase enzyme (Cox-1) and preventing thromboxane A$_2$ (TXA$_2$) formation. GPIIb-IIIa inhibitors block the final step of platelet aggregation common to all agonists, the binding of fibrinogen or vWF to activated GPIIb-IIIa complexes. Such inhibitors include an antibody, abciximab (c7E3 Fab fragments), a cyclic peptide (epitifibatide), and 2 peptidomimetics (lamifiban and tirofiban), all of which require intravenous injection (left side of Figure). Orally bioavailable inhibitors include xemilofiban, DMP 802, and SR 121787 (right side of Figure). These are just selected examples of the anti-integrin therapies being tested.

cases by tissue plasminogen activator or even by PTCA by halting the incorporation of incoming platelets. However, the association of abciximab or other anti–GPIIb-IIIa agents with powerful anticoagulants or with thrombolitics increases the risk of bleeding.

The fabrication of synthetic small-molecule inhibitors (some are called peptidomimetics because they mimic RGD peptides) designed for intravenous administration, such as epitifibatide (integrilin), lamifiban, and tirofiban, means that alternative anti–GPIIb-IIIa therapies are now available. Eptifibatide is a small cyclic heptapeptide, and lamifiban and tirofiban are nonpeptide peptidomimetics. These compounds, which circulate for shorter times than does abciximab, have also been found to be beneficial in acute situations, such as after PTCA or stenting. Another family of synthetic inhibitors, such as xemilofiban, DMP 802, and SR 121787, may be taken orally and are being assessed for long duration use in patients with coronary artery disease who are considered vulnerable for major thrombotic episodes. This latter group consists mostly of prodrugs that are biologically transformed into active metabolites in the vessel wall or to a fibrin clot or by soluble agonists such as ADP and thrombin. Antiplatelet therapies can target individual receptor-linked activation pathways. For example, ticlopidine and clopidogrel act on an ADP-induced activation pathway. Aspirin dampens the platelet response by inactivating cyclooxygenase enzyme (Cox-1) and preventing thromboxane A$_2$ (TXA$_2$) formation. GPIIb-IIIa inhibitors block the final step of platelet aggregation common to all agonists, the binding of fibrinogen or vWF to activated GPIIb-IIIa complexes. Such inhibitors include an antibody, abciximab (c7E3 Fab fragments), a cyclic peptide (epitifibatide), and 2 peptidomimetics (lamifiban and tirofiban), all of which require intravenous injection (left side of Figure). Orally bioavailable inhibitors include xemilofiban, DMP 802, and SR 121787 (right side of Figure). These are just selected examples of the anti-integrin therapies being tested.

Effect on Platelet Activation
Platelet activation is known to occur episodically in coronary syndromes, and elevated levels of circulating activated platelets, as detected by flow cytometry, have been said to be predictive for increased risk of acute ischemic events after PTCA or stenting, although the interpretation of such measurements remains controversial. We have recently compared the expression of activation-dependent markers on platelets during abciximab therapy given according to the CAPTURE protocol to patients with unstable angina undergoing PTCA. Before the onset of therapy, the percentage of...
platelets positive for one or more of the monoclonal antibodies directed against GPIIb-IIIa complexes that are “activated and unoccupied” (PAC-1) or “activated and occupied with fibrinogen” (AP6 and F26) were elevated for 5 of 6 patients studied. Abciximab therapy reduced these levels appreciably, presumably through the blockade of the active site of GPIIb-IIIa.

Testing of samples taken after the bolus and 3 hours into the infusion confirmed that for most patients near-maximal inhibition of platelet aggregation was already achieved. This inhibition continued during the duration of the abciximab infusion (up to 24 hours). However, for one patient, a residual irreversible aggregation response was seen with ADP. Interestingly, it was for this patient that activation-dependent markers on GPIIb-IIIa continued to be detected, suggesting continued platelet hyperactivity and a resistance to abciximab.

Peter et al. have recently shown that the binding of abciximab itself induced conformation changes in GPIIb-IIIa and, in so doing, mimicked the changes previously shown for some RGD peptides. According to these authors, the changes were such that when abciximab dissociated from the complex, a process facilitated in their experimentation by the large-scale dilution of the platelet suspensions, fibrinogen was able to bind without the normal requirement for platelet stimulation by an agonist. Peter et al. also showed that 2 small-molecular-mass inhibitors were able to activate GPIIb-IIIa but that aspirin prevented “aggregation” induced by the anti–GPIIb-IIIa drugs. Further studies are required to confirm that such events occur in vivo and to determine whether this can lead to clinical drawbacks. In our previous study, activated platelets progressively reappeared in the circulation after the stopping of the abciximab, but this can be due to the fact that the cause of increased platelet stimulation had not been removed (eg. fissured atherosclerotic plaques, stenosis, and resistant fibrin-rich thrombi).

Intersubject heterogeneity in the extent and duration of inhibition of platelet aggregation has been previously shown in patients with coronary artery disease receiving abciximab. Among the other factors that may influence the recovery rate after anti–GPIIb-IIIa therapy are (1) variations in the plasma levels of free drug, (2) the rate at which surface-bound drug dissociates from circulating platelets, and (3) the extent to which GPIIb-IIIa receptors are exchanged between surface and internal pools. Therefore, it is reasonable to suggest that biological surveillance, with testing of platelet function and flexibility with regard both to the doses used and the duration of therapy, may help to improve the success rate with anti–GPIIb-IIIa inhibitors in acute situations. This may also be so with orally bioavailable drugs that are used chronically and for which the degree of sustained receptor inhibition may be lower.

Internal Pools of GPIIb-IIIa and Trafficking of Abciximab

Although ADP-induced platelet aggregation was extensively inhibited by abciximab in our previous study, platelet responses to thrombin receptor–activating peptide (14mer) during abciximab infusion ranged from 28% to 71% of pretreatment levels. Similar results have previously been reported for patients after a single bolus injection of abciximab. Estimates suggest that between 50 000 and 100 000 copies of GPIIb-IIIa are to be found on the surface of resting platelets and that a pool of similar proportions is to be found inside the platelet (data reviewed in Reference 30). Using the monoclonal antibody AP-2, competitive for GPIIb-IIIa with c7E3, in flow cytometry or immunoelectron microscopy, we have shown that unblocked (with abciximab) GPIIb-IIIa receptors from the internal pool do indeed become exposed after thrombin stimulation of platelets taken from patients during abciximab infusion. A residual aggregation with thrombin receptor–activating peptide during the infusion and after the administration of the abciximab bolus suggests that the concentration of free abciximab in plasma is insufficient to block these newly exposed receptors within the time scale of platelet aggregation, which will occur in seconds. The ability of peptides, peptidomimetics, and orally bioavailable drugs to inhibit aggregation with strong agonists will therefore depend on their affinity for GPIIb-IIIa and their sustained concentration in plasma. Furthermore, some GPIIb-IIIa complexes of the internal pool may be surface-expressed with the already active site occupied with fibrinogen being secreted from α-granules, as has been shown for platelets stimulated in vitro with thrombin.

The ability of anti–GPIIb-IIIa drugs to gain access to internal pools of GPIIb-IIIa complexes of circulating platelets is therefore an important question to address. We have examined the trafficking of abciximab within platelets at different time points during therapeutic infusion by immunoelectron microscopy using a rabbit antibody specific for c7E3. It appears that abciximab gains access to the internal membrane subpopulations of GPIIb-IIIa by 2 mechanisms: (1) through thin channels of the surface-connected canalicular system and (2) by way of clathrin-coated pits and endocytosis. However, there is not a continuous accumulation and storage of the antibody within α-granules as is seen, for example, for fibrinogen. This means that internal pools of GPIIb-IIIa do not become saturated with the drug. Furthermore, the fact that abciximab is a Fab fragment and monovalent raises the possibility that we are visualizing natural recycling of GPIIb-IIIa between the surface and internal pools. Such recycling has previously been implied from studies performed with an RGD-based peptide; thus, small-molecular-mass inhibitors of GPIIb-IIIa may be expected to behave similarly. The difference in the functional response between platelets from patients receiving abciximab and those from patients with Glanzmann’s thrombasthenia is important to emphasize. In the latter, an inherited GPIIb-IIIa deficiency extends to all membrane systems, whereas at the present therapeutic doses of abciximab, a residual pool of functional GPIIb-IIIa complexes can be expressed after a strong hemostatic challenge. Whether this limits the efficacy of abciximab or provides a protective backup to limit the tendency for hemorrhage is an essential question to address both for abciximab and for other anti–GPIIb-IIIa drugs.

Interindividual Variability in the Response to Treatment

The inhibition provided by abciximab continues at various levels for several days after the infusion has stopped. Possible explanations for its durability include the combined effects of a high affinity for GPIIb-IIIa and a frequent association/
Some of the Factors That May Influence the Efficacy of GPIIb-IIIa Inhibitors in Different Individuals

1. Platelet count
   - High: low level of GPIIb-IIIa occupancy
   - Low (including enlarged spleen): increased bleeding risk
2. Plasma antibodies
   - To mouse immunoglobulin determinants (abciximab)
   - To neogeotgens on GPIIb-IIIa (type anti-LIBS)
3. Presence of circulating activated platelets
4. Capacity for the inhibition of the internal pool of GPIIb-IIIa complexes
   - In circulating resting platelets
   - During secretion and surface expression
5. Interindividual variations in the reactivity of the drug with GPIIb-IIIa
   - Polymorphisms of GPIIb and/or GPIIIa
   - Influence of high or low GPIIb-IIIa density
6. Different rates of drug metabolism or in the rate of elimination from the bloodstream
7. Presence of other thrombotic risk factors (eg, high cholesterol may influence membrane fluidity; diabetes, factor V Leiden, or prothrombin gene G 20210 A variants, polymorphisms of other platelet receptors)
8. Degree of atherosclerosis or variations in the size of the PTCA lesion and the frequency of platelet/vessel wall interactions
9. Variations in the reactivity of platelets with other drugs used during therapy (eg, aspirin, heparin)

LIBS indicates ligand-induced binding sites.

Many of the factors described above are not unique to patients treated with GPIIb-IIIa inhibitors. Other variables include variations in patient reactivity to heparin or other antiplatelet drugs, such as aspirin or calcium antagonists, in the presence of circulating activated platelets, or in the capacity for the inhibition of the internal pool of GPIIb-IIIa complexes.

It is plausible that some individuals possess GPIIb-IIIa complexes, where abciximab (or other anti-GPIIb-IIIa drugs) bind with an altered affinity or where there is an increased propensity for fibrinogen to bind (Table). Polymorphisms in the cytoplasmic domains of GPIIb and GPIIIa, implicated in controlling the activation state of the complex, are a potential field for study here. Furthermore, a higher than usual density of GPIIb-IIIa complexes could render a standard dose of drug unsuccessful. Variations in patient reactivity to heparin or other medications received by patients with unstable angina (nitrates, calcium antagonists, and/or β-blockers) may influence the end result. The activating potential of heparin on platelets is well known, and its ability to potentiate the expression of activation-dependent markers has recently been reported. Whereas there is a standard dose of drug, there is no such thing as a standard patient. Thus, increased biological testing to assess whether platelet function is adequately inhibited might improve the success rate with all anti–GPIIb-IIIa drugs. Although platelet aggregometry or flow cytometry are of proven use, simple and rapid point-of-care tests may provide the answer. Among such tests is an automated and quantitative cartridge-based method assessing the competence of the GPIIb-IIIa receptor, as reflected in the ability of platelets to agglutinate fibrinogen-coated beads. An alternative system looks at the activated clotting time of whole blood.

Other Potential Actions of GPIIb-IIIa Inhibitors That Influence Their Activity

Abciximab not only reacts with GPIIb-IIIa but also demonstrates equivalent affinity and functional blockade of the αvβ3 integrin. This latter integrin is widespread, being found on endothelial cells, osteoclasts, and smooth muscle cells, among others. We have recently confirmed the reactivity of this drug with the luminal surface of endothelial cells lining the vascular sinuses in the bone marrow (C. Poujol, C. Durrieu-Jais, B. Larrue, A.T. Nurden, P. Nurden, unpublished data, 1999). Abciximab also interacts with an activation-dependent neoantigen present on the leukocyte integrin Mac-1. These findings are explained through the presence of subunit structural homology and a common epitope recognized by the antibody, which blocked the binding of Mac-1–bearing cells to fibrinogen and intercellular adhesion molecule-1, both ligands of Mac-1. The implication is that the drug can interfere with the recruitment of monocytes to sites of vessel injury and inflammation. Although whether these properties provide an additional in vivo benefit of abciximab therapy remains controversial, an inhibitory action of antiplatelet drugs on Mac-1–bearing cells to fibrinogen and intercellular adhesion molecule-1, both ligands of Mac-1. The finding by Reverter et al that abciximab has a dampening effect on platelet-mediated thrombin generation may also help explain its antithrombotic efficacy. In that study, the evidence from in vitro experiments suggested that blocking ligand binding to GPIIb-IIIa and αvβ3 on platelets can inhibit tissue factor–induced thrombin generation by up to 50%. This was caused by inhibition of the expression of procoagulant activity on platelets and the release of procoagulant microparticles (surface exposure of aminophospholipids is followed by a Ca2+-dependent binding of factor Va, factor Xa, and prothrombin and a process of microvesiculation). This unexpected dampening of platelet reactivity may be due to the inhibition of “outside-in” signaling promoted by GPIIb-IIIa occupancy and platelet aggregation. Prothrombin has also been shown to bind to GPIIb-IIIa directly but by a mechanism different from that of fibrinogen. Reduced thrombin generation in the area of vessel injury may also mean less smooth muscle migration and hyperplasia (and restenosis), and less fibrin-bound thrombin within the clot can reduce resistance to thrombolysis via activation of factor XIII. Finally, there is evidence that fibrin can also interact with
additional sites on GPIIb-IIIa complexes compared with soluble fibrinogen. Because fibrin is a component of most thrombi, the ability of drugs to inhibit platelet attachment to fibrin may be another factor in controlling their antithrombotic potential. Thus, there is a great deal yet to learn about the mode of action of anti–GPIIb-IIIa drugs, and new inhibitors must be tested as comprehensively as possible. Not least, our understanding of the mechanisms underlying those ischemic events that continue despite the use of the anti–GPIIb-IIIa inhibitors must be increased, because these drugs, although representing a major step forward in the control of arterial thrombosis, do not provide a total protection.

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References


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