Glycoprotein IIb/IIIa receptor and its inhibition: A platelet-directed therapeutic strategy

H. D. Shah, R. K. Goyal

ABSTRACT

Platelets play a key role in atherosclerosis, thrombosis and acute coronary syndromes. Drugs that dissolve blood clot (thrombolytic agents) and that prevent clot propagation (antiplatelet and anticoagulant agents) are used to treat a broad array of cardiovascular diseases. Therapeutic manipulation of platelet function has focused principally on the use of aspirin which has proved effective in many clinical situations, despite its relatively weak antiplatelet action as compared to newer agents like ticlopidine, clopidogrel and more recently, platelet glycoprotein (GP) IIb/IIIa receptor inhibitors. The platelet GP IIb/IIIa receptor has been identified as a pivotal mediator of platelet aggregation, making it a logical target for the control of the platelet response to vascular injury. The primary mechanism of GP IIb/IIIa antagonists is the inhibition of the final common pathway of platelet aggregation: fibrinogen binding to the GP IIb/IIIa complex. Various antagonists of the GP IIb/IIIa receptor are currently receiving considerable attention and are being investigated for various clinical settings including angina, myocardial infarction and interventional cardiology.

KEY WORDS: Antiplatelet agents, antithrombotics, coronary artery disease, glycoprotein IIb/IIIa antagonists

It is well known that platelets play a central role in acute coronary syndromes. Understanding the molecular mechanism that underlies the pathophysiology of acute coronary syndromes has been critical in developing optimal pharmacological therapies. Given the key role of thrombosis, pharmacological therapy has sought to provide potent inhibition of both platelet aggregation and coagulation cascade. Traditionally, antiplatelet therapy with aspirin and anticoagulation with Unfractionated Heparin (UFH) has been the cornerstone of the management of patients with cardiovascular diseases. Aspirin, however, blocks only one of the several signal transduction pathways leading to platelet activation (i.e. inhibits prostaglandin G/H synthase). Therefore, platelet activation and aggregation is effectively allowed to continue even in its presence. UFH binds to antithrombin III, thus inhibiting the activity of the coagulation cascade. However, UFH is ineffective against clot-bound thrombin. Low molecular weight heparin (LMWH) offered several potential advantages over traditionally used UFH, including minimal interaction with platelets and lower incidences of heparin-induced thrombocytopenia. Additionally, unlike UFH, LMWHs do not interact with plasma proteins, hence their anticoagulant effects are more predictable, eliminating the need for monitoring. However, it is a matter of concern that platelet aggregation and development of thrombosis are relatively resistant to conventional therapy with fibrinolytics. Newer agents like ticlopidine and clopidogrel were developed initially as an adjunct to aspirin. The antiplatelet action of ticlopidine is principally exerted by blocking the ADP-mediated activation of platelet glycoprotein (GP) IIb/IIIa receptors. Clopidogrel is a newer thienopyridine derivative, chemically related to ticlopidine. It also blocks activation of GP IIb/IIIa receptors by irreversibly inhibiting the binding of the agonists to their receptors on the platelets, thereby affecting the ADP-dependent activation of the GP IIb/IIIa complex. Platelet aggregation involves cross-linking of activated glycoprotein (GP) IIb/IIIa receptors on two adjacent platelets by a single molecule of plasma fibrinogen. The platelet GP IIb/IIIa receptor has been identified as a target for control of the platelet response to vascular injury. During the last decade intensive efforts have been made to evaluate the role of the GP IIb/IIIa complex in platelet-mediated thrombus formation. GP IIb/IIIa serves as the receptor on platelets that binds plasma-borne adhesive proteins, such as fibrinogen and von Willebrand factor; thus permitting platelet aggregation. GP IIb/IIIa receptor antagonists act by inhibiting this final common pathway of platelet aggregation.
have summarized the role of GP IIb/IIIa in platelet function, its antagonists and their application as newer and safe therapeutic agents.

**Biology of platelet function**

*Platelet adhesion and aggregation*

Platelet adhesion, the first step in the process of hemostasis, is triggered by damage to the vessel wall and local exposure of the sub-endothelial matrix. It involves deposition of platelets from the circulation onto the newly exposed sub-endothelial surfaces and occurs almost instantaneously after vascular and tissue trauma that leads to interruption of the continuity of blood vessels. Local mechanical or biochemical stimuli can induce activation of adherent platelets. High shear blood flow condition can activate adherent platelets even in the absence of additional stimuli, but the process is more rapid when biochemical stimuli are involved. Molecules secreted or released from platelets include platelet activators such as thromboxane A₂ (TXA₂), serotonin, ADP and epinephrine, and mediators of inflammation and vascular repair such as cluster determinant (CD) 40 ligand, platelet derived growth factor (PDGF)-β and tumor growth factor (TGF)-β. Platelet activation is also characterized by changes in the platelet surface that promote coagulation and aggregation. Regardless of the mode of platelet activation, platelet aggregation is mediated by the GP IIb/IIIa receptor and it is the final common pathway in thrombus formation. Figure 1 depicts the role of GP IIb/IIIa in platelet aggregation. Unstimulated discoid platelets are shown with receptors for thrombin, ADP, collagen, vWF and immobilized fibrinogen. All of these are capable of stimulating platelets, inducing change in platelet shape, and activating the receptor function of GP IIb/IIIa.

Platelet adhesion and aggregation are controlled by the activity of the platelet membrane receptors which biochemically are GPs. According to their genetic origin, GPs can be divided into five groups called platelet gene families: the integrins, the leucin-rich GP family, the selectin family, the quadraspanin family and the immunoglobulin supergene family. The thrombus formation initiated by platelet adhesion to extracellular matrix (ECM) involves the synergistic function of at least four receptors, the GP Ib-IX-V complex and the integrins α₃β₁ (GP Ia-IIa), α₅β₃ (GP IIb-IIIa) and α₇β₃ (GP Ic-IIIa). Aggregation, in contrast, may depend only on the GP Ib-IX-V complex and α₅β₃ (Table 1). To form stable bonds either with the ECM components or with other platelets, circulating platelets must attach to a reactive substrate, resisting the force of flowing blood, which would tend to move platelets with the layer of fluid adjacent to the vessel wall. This reactive substrate is provided by sub-endothelial and extra-vascular ECM components during adhesion, and during aggregation activated platelets that are already firmly adherent play this role; but in either case, fluid drag opposes the initial establishment and subsequent enlargement of the thrombus. Interaction proposed to mediate platelet adhesion and aggregation during thrombus formation is depicted in Figure 2.

**GP IIb/IIIa receptor and its role in platelet aggregation**

The GP IIb/IIIa receptor (α₃β₃) belongs to the integrin family of platelet receptors. Its structure has been shown in the Figure 3. The receptor consists of two subunits, alpha and beta. The alpha subunit is of 136-kDa and consists of a heavy chain and a light chain. The light chain has a short cytoplasmic tail, a transmembrane region, and a small extracellular domain; whereas the heavy chain is entirely extracellular. The beta

![Figure 1: Overview of the processes of platelet activation and aggregation as well as inhibition of platelet aggregation by glycoprotein (GP) IIb/IIIa receptor antagonists. Numerous platelet agonists like adenosine diphosphate, thrombin, collagen and epinephrine can promote activation of platelets.](image-url)
Table 1:
Platelet-membrane glycoprotein receptors involved in the adhesion and aggregation of platelets

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Receptor-mediated action</th>
<th>Amino acid sequence recognized</th>
</tr>
</thead>
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<tr>
<td><strong>Integrin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\alpha_2\beta_1$ (glycoprotein Ia/IIa)</td>
<td>Collagen</td>
<td>Adhesion</td>
<td>DGEA*</td>
</tr>
<tr>
<td>$\alpha_5\beta_1$ (glycoprotein Ic/IIa)</td>
<td>Fibronecin</td>
<td>Adhesion</td>
<td>RGD</td>
</tr>
<tr>
<td>$\alpha_6\beta_1$</td>
<td>Laminin</td>
<td>Adhesion</td>
<td>Not confirmed to a short sequence</td>
</tr>
<tr>
<td>$\alpha_\text{Ibb}_\beta_3$ (glycoprotein IIba/IIIa)</td>
<td>Fibrinogen</td>
<td>Aggregation</td>
<td>KQAGDV or RGD</td>
</tr>
<tr>
<td>$\alpha_\text{Ibb}_\beta_3$ (glycoprotein IIba/IIIa)</td>
<td>Fibronecin</td>
<td>Aggregation</td>
<td>RGD*</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>Vitronecin</td>
<td>Aggregation</td>
<td>RGD</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>Adhesion</td>
<td></td>
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<tr>
<td>$\alpha_\text{Vbb}_\beta_3$</td>
<td>Vitronectin</td>
<td>Adhesion</td>
<td>RGD</td>
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<td>$\alpha_\text{Vbb}_\beta_3$</td>
<td>Fibrinogen</td>
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<td>$\alpha_\text{Vbb}_\beta_3$</td>
<td>Fibronectin- von</td>
<td>Adhesion</td>
<td>RGD</td>
</tr>
<tr>
<td>$\alpha_\text{Vbb}_\beta_3$</td>
<td>Factor (vWf)</td>
<td>Adhesion</td>
<td>RGD</td>
</tr>
<tr>
<td>Non- Integrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycoprotein Ib</td>
<td>von Willebrand factor</td>
<td>Adhesion</td>
<td>Not confirmed to a short sequence</td>
</tr>
<tr>
<td>Glycoprotein IV</td>
<td>Thrombospondin</td>
<td>Adhesion</td>
<td>CSVTCG</td>
</tr>
<tr>
<td>Collagen</td>
<td>Adhesion</td>
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</tbody>
</table>

* Other amino acid sequences may also be involved.

subunit is of 92 kDa and consists of a single polypeptide of 762 amino acids, with a short cytoplasmic tail, a single transmembrane region, and a large extracellular domain. There are various types of platelet gene families and their subtypes. Accordingly, there are different types of receptors and their subunits (alpha and beta) differ in structure and combinations. Among various integrins, the $\alpha_{\text{Ibb}}$ sub-unit has been found in combination with $\beta_3$, and this is found to be present in the cells of megakaryocyte lineage. Both the alpha and beta subunits are non-covalently bound to each other, and calcium is required to maintain the heterodimeric structure. Platelet activation causes changes in the shape of platelets and conformational changes in GP IIb/IIIa receptors, transforming the receptors from a ligand-unreceptive to a ligand-receptive state (Figure 3). Ligand-receptive GP IIb/IIIa receptors bind fibrinogen molecules, which form bridges between adjacent platelets and facilitate platelet aggregation. Inhibitors of GP IIb/IIIa receptors also bind to these receptors, blocking the binding of fibrinogen and thus preventing platelet aggregation. Although the binding of fibrinogen to GP IIb/IIIa receptors is the principal mechanism for platelet aggregation, other adhesive GPs including fibronectin, von Willebrand factor (vWf) and vitronectin also bind to these receptors. Much effort has been directed towards characterizing the molecular basis for the binding of the soluble adhesive proteins vWF and fibrinogen to platelets.

![Figure 2](image2.png)

**Figure 2:** Interaction proposed to mediate platelet adhesion and aggregation during thrombus formation: Activation usually precedes stable adhesion, particularly when thrombus formation occurs under the influence of high shear stress, but specific adhesive bonds may also enhance activation.

![Figure 3](image3.png)

**Figure 3:** Structure of platelet glycoprotein IIb/IIIa receptor.
specificity of the GP IIb/IIIa receptor is defined by two peptide sequences, through which the agonists bind to it. One is the Arg-Gly-Asp (RGD) and another sequence is Lys-Gln-Ala-Gly-Asp-Val (KQ/AGDV). The RGD sequence was initially identified as the adhesive sequence in fibronectin but it is also present in fibrinogen, von Willebrand factor and vitronectin. All these ligands contain at least one RGD sequence, whereas fibrinogen contains two RGD sequences per half molecule. The KQ/AGDV sequence is the other major sequence involved in the binding of fibrinogen to GP IIb/IIIa receptors and is located at the carboxyl terminus of the γ-chain of fibrinogen. Unlike RGD, this sequence is found only in fibrinogen and is probably the predominant site for the binding of fibrinogen to GP IIb/IIIa receptors. Apart from fibrinogen binding, GP IIb/IIIa receptors on unstimulated platelets are also involved in binding of prothrombin, an interaction that increases the rate of prothrombin conversion to thrombin.

Since the GP IIb/IIIa receptor is the final common pathway by which platelet aggregation takes place, direct inhibition of this receptor is likely to prove superior to blockers of only some of the pathways. Various antagonists of GP IIb/IIIa are currently receiving considerable attention from the pharmaceutical industry and are being studied in a variety of clinical settings. Significant efforts have also been made to design potent antagonists of this final common pathway of platelet aggregation to be used as novel therapeutic strategies to inhibit thrombosis that leads to acute coronary syndromes.

Although several different GP IIb/IIIa antagonists have convincingly demonstrated the usefulness of this platelet-directed therapeutic strategy, a number of unsolved and sometimes misunderstood issues concerning the pharmacology and optimal clinical usefulness of these agents remain to be explored.

**GP IIb/IIIa receptor antagonists**

GP IIb/IIIa antagonists belong to an unusual drug class, in part because platelet stimulation, which occurs during arterial thrombosis, may change the number of functional GP IIb/IIIa complexes on the platelet surface and also because the receptor cannot be saturated, but rather is titrated to optimize antithrombotic activity and minimize antihemostatic activity. Potent agonists such as thrombin can cause up to a 50% increase in the number of GP IIb/IIIa molecules exposed on the platelet surface; therefore, receptor occupancy after stimulation becomes an important consideration to predict antithrombotic efficacy.

The first GP IIb/IIIa antagonist developed for clinical investigation was the murine monoclonal antibody M7E3. M7E3 demonstrated prevention of platelet aggregation by inhibiting fibrinogen binding. Later abciximab, a Fab chimera that retains the mouse-derived variable portion of M7E3 joined the constant region of human IgG Fab. It has undergone extensive clinical evaluation and is approved by regulatory agencies worldwide as an adjunct to coronary intervention. Natural products have also been screened extensively for activity against GP IIb/IIIa receptors. Trigramin, isolated from the venom of the viper Trimeresurus gramineus, is a potent inhibitor of ligand binding to the GP IIb/IIIa receptors. Synthetic linear peptides based on the RGD template have also been investigated. These include agents like G 4120 and MK-856. However, these had relatively little activity and were more resistant to enzymatic degradation. Replacement of the arginine group in the RGD sequence with an amidino or benzamidino containing group increased the resistance to enzymatic degradation. Agents in this group include lamifiban (previously known as Ro 44-9883) and tirofiban (previously known as MK-383). A novel non-peptide GP IIb/IIIa antagonist XV 4594 and another long acting potent fibrinogen receptor antagonist L-738,167 are being tested for their antithrombotic efficacy. However, the four clinically used parenteral GP IIb/IIIa antagonists include abciximab, the cyclic peptide epifibatide, the non-peptide tirofiban, and the peptidomimetic lamifiban.

Oral GP IIb/IIIa antagonists are being investigated for secondary prevention of cardiovascular morbidity and mortality. These include xemilofiban, orbofiban, sibrafiban (SC-54684) and roxifiban. Roxifiban has been tested in the Roxifiban Oral Compound Kinetic Evaluation Trial (ROCKET-I) for its effect on platelet aggregation and major receptor expression in patients with CAD.

**Properties**

Although all GP IIb/IIIa inhibitors have a rapid onset of action, the return of platelet function to normal following drug discontinuation varies according to the individual agent. The duration of the action of epifibatide and tirofiban is short because they bind reversibly to the GP IIb/IIIa receptor and have a short half-life. Therefore, within a relatively short time after the discontinuation of these agents, there is little or no increased risk of bleeding. Abciximab has a short plasma half life but a long duration of action due to its high affinity binding to the receptors. Both epifibatide and tirofiban HCI are highly specific for the platelet receptor GP IIb/IIIa, whereas abciximab also binds to the related integrins vitronectin and Mac-1. Due to its relatively large size and murine origin, abciximab is capable of eliciting an antibody response, which has not been observed with either epifibatide or tirofiban HCI. The P1A2 genotype of the GP IIb/IIIa receptor appears to be associated with adverse outcome in patients treated with oral GP IIb/IIIa antagonists. Thus, P1A2 polymorphism of these receptors is now emerging as a probable determinant of the response to antiplatelet agents. Soluble CD40 ligand (sCD40L) is a prothrombotic and proinflammatory protein that is released by platelet activation and subsequent aggregation by GP IIb/IIIa activity. Therefore, GP IIb/IIIa antagonists may not only inhibit thrombosis through blockade of platelet aggregation but may also inhibit inflammation and thrombosis through blockade of sCD40L release.

**Clinical trials overview**

**GP IIb/IIIa receptor antagonists in interventional cardiology**

Five large, randomized, placebo controlled trials of GP IIb/IIIa antagonists define our current knowledge regarding the adjunct use of these agents during coronary intervention. These include EPIC (Evaluation of M7E3 for the Prevention of Ischemic Complication) trial, EPILOG (Evaluation in PTCA to
Improve Long term Outcome with abciximab GP IIb/IIIa Blockade) trial, CAPTURE (c7E3 Fab Antiplatelet Therapy in Unstable Refractory angina) trial, IMPACT-II (Integrin [eptifibatide] to Minimize Platelet Aggregation and Coronary Thrombosis II) trial and RESTORE (Randomized Efficacy Study of Tirofiban for Outcome and Restenosis) trial. In each trial the study was initiated before coronary intervention. The EPIC trial showed conclusively that administration of abciximab in the form of a bolus followed by a low dose infusion for 12 h produced significant reduction in 30 day combined primary endpoints (death, non-fatal myocardial infarction, C A B G or emergency PTCA, stent insertion for procedural failure and balloon pump insertion for refractory angina). The EPILOG trial compared abciximab and standard dose heparin (100 U/kg bolus and additional to maintain ACT>300s), with abciximab and low dose heparin (70 U/kg bolus and ACT>200s) and with placebo and standard dose of heparin. At the end of 30 days, composite endpoints like death, myocardial infarction (MI) and revascularisation were significantly reduced in low dose heparin with abciximab group as well as in standard dose heparin with abciximab group. The CAPTURE trial provided evidence that, in patients with refractory unstable angina, pretreatment with abciximab may reduce the incidence of MI prior to PTCA.

The 30 day results of the RESTORE trial showed that tirofiban produced a decrease in composite endpoint of death, MI, C A B G, Target vessel revascularisation (T V R) or stent insertion for threatened closure. The IMPACT-II trial compared a low dose infusion regime of eptifibatide (0.5 μg/kg/min) with a high dose infusion (0.75 μg/kg/min) as well as with placebo. The results suggested that the low dose infusion was better than the high dose infusion regime. However, there was no difference between the low dose infusion regime and the placebo group in the composite end points (death, MI, urgent revascularization by PTCA or C A B G, or stent replacement for abrupt closure). The recently published Evaluation of Platelet Inhibitor for Stenting (EPISTENT) trial compared the use of abciximab in conjunction with angioplasty or stenting without abciximab. The 30 day primary end points of death, MI or urgent revascularization were reduced to a great extent in patients receiving stenting plus abciximab as compared to angioplasty or abciximab alone group. It has been established that abciximab and tirofiban can be used successfully in patients with peripheral arterial occlusion disease and arterial thrombosis.

**GP IIb/IIIa inhibitors in acute myocardial infarction**

The majority of trials previously mentioned included a small number of patients with acute myocardial infarction (AM I). Until recently, overriding concern about the potential for intracranial hemorrhage induced by GP IIb/IIIa inhibition coupled with heparin, aspirin and thrombolytic therapy has precluded the large-scale investigation of GP IIb/IIIa antagonists in acute MI. Regarding the utility of GP IIb/IIIa inhibition with direct angioplasty, the RAPPORT (Reopro in acute myocardial infarction and primary PTCA organization and Randomized Trial) showed a significant reduction in the rates of urgent TVR, particularly during the first seven days. The Controlled Abciximab and Device Investigation to Lower Late angioplasty Complication (CADILLAC) trial suggested benefit of abciximab with both primary PTCA and primary stenting but no additional benefit of the combined use of abciximab and stents.

Another one, the Abciximab before Direct angioplasty and stenting in Myocardial Infarction regarding Acute and Long term follow-up (ADMIRAL) trial is also looking at the long term effects of abciximab and primary stenting.

**GP IIb/IIIa inhibitors in unstable angina or non Q wave MI**

GP IIb/IIIa receptor inhibitors are beneficial in unstable angina/ non-ST elevation MI. Four large, randomized, placebo controlled trials evaluated parenteral GP IIb/IIIa antagonists in this syndrome. These included PRISM (Platelet Receptors inhibition in Ischemic Syndrome Management) trial, PRISM-PLUS (Platelet Receptors inhibition in Ischemic Syndrome Management in Patients Limited by Unstable signs and Symptoms) trial, PARAGON (Platelet IIb/IIIa Antagonism for the Reduction of Acute coronary syndrome events in a Global Organization Network) trial and PURSUIT (Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression using Integrilin [eptifibatide] Therapy) trial. The PRISM trial randomized patients with unstable angina/non-ST elevation MI to intravenous heparin or intravenous tirofiban for 48 h. It showed a composite endpoint reduction in favor of tirofiban. The PRISM-PLUS trial was designed to assess whether tirofiban with heparin was better than either drug alone in the management of a similar group of patients as the PRISM trial. The tirofiban arm was terminated prematurely owing to excess mortality. However, combination of tirofiban and heparin produced highly significant reduction in 7 day composite endpoint (death, MI or refractory angina). In the PARAGON trial, another peptide GP IIb/IIIa antagonist lamifiban, in low and high dose was compared, with or without heparin. No significant benefit was however obtained in these trials. The PURSUIT trial is by far the largest trial of GP IIb/IIIa therapy with 10948 patients showing eptifibatide to produce significant reduction in incidences of death or MI at 30 days in those undergoing PTCA.

However, the main disadvantages of the use of GP IIb/IIIa inhibitors are an increased tendency to bleeding, thrombocytopenia and perhaps its cost as well. The determination of an appropriate degree of GP IIb/IIIa blockade with agents that will be both effective and safe is perhaps the greatest challenge in the development of oral agents. The other major challenge in the development of oral antagonists will be the nature of the pharmacokinetic and pharmacodynamic variability that they display in patients.

To conclude, the GP IIb/IIIa receptor is a unique target in cardiology for the prevention of restenosis. Various clinical trials have convincingly established that GP IIb/IIIa receptor antagonists produce a significant reduction of death, non-fatal myocardial infarction, emergency C A B G or PTCA, stent or balloon pump insertion for refractory angina. More comparative studies are still required to prove the efficacy and safety of these drugs.

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