

Platelet Membrane Glycoproteins

Gilbert C. White II

Platelet adhesion and aggregation are mediated by protein receptors on the platelet surface. Recent advances in our understanding of the structure and function of these platelet surface receptors have led to the development of new and powerful methods for regulating platelet function that promise to be of great benefit in the treatment and, perhaps, prediction of vascular diseases. This review will focus on these membrane proteins and the ongoing work to target them for therapeutic intervention.

Glycoproteins IIb and IIIa (aIIbb3)

The glycoprotein (GP) IIb/IIIa complex is an abundant platelet surface receptor which mediates platelet aggregation when GPIIb/IIIa on one platelet binds fibrinogen or fibrin, which, by virtue of its dimeric structure, interacts with GPIIb/IIIa on another platelet. GPIIb/IIIa is also able to bind von Willebrand factor, vitronectin, and fibronectin. Members of the integrin family of proteins, GPIIb and IIIa, are integral membrane proteins that form a calcium-dependent heterodimer complex on the platelet surface. On resting platelets, GPIIb/IIIa is in an inactive state and is unable to bind fibrinogen/fibrin. Following platelet activation, GPIIb/IIIa becomes activated through a process that involves protein kinase C, heterotrimeric G proteins, and calcium. Activation of GPIIb/IIIa appears to be a multi-step process which initially involves induction of the receptor followed by reversible fibrinogen binding followed by irreversible fibrinogen binding.

In general, the recognition of ligand by integrin receptors is through a tripeptide arginine-glycine-aspartic acid (RGD) sequence in the ligand. However, fibrinogen binding to GPIIb/IIIa is mediated by the carboxy-terminal dodecapeptide sequence, HHLGGAKQAGDV, in the γ -chain of fibrinogen where the AGDV sequence has been suggested to have structural similarity to RGD. Interestingly, there are two RGD sequences in fibrinogen, RGDS at amino acids 95-98 and RDGF at amino acids 572-575 in the A α chain. Although neither appears to mediate the interaction with fibrinogen, the RGD sequences may play a role in the interaction of GPIIb/IIIa with fibrin. Thus, although fibrinogen and fibrin are closely related molecules, the interaction of each with GPIIb/IIIa differs. Fibrinogen binding requires activation of GPIIb/IIIa and is mediated through the γ -chain dodecapeptide, while fibrin binding does not appear to require activation of GPIIb/IIIa and may utilize the A α chain RGD sequences. Fibrinogen/fibrin binding to GPIIb/IIIa can be inhibited by small peptides containing the RGD sequence, but these peptides also inhibit, to greater or lesser degree, the interaction of other integrins with their ligands. The specificity of peptide inhibition of ligand binding resides in

amino acids flanking the RGD sequence. Fibrinogen/fibrin binding to GPIIb/IIIa can also be inhibited by monoclonal antibodies, such as 7E3 and AP2, that interact with and block the ligand binding site of GPIIb/IIIa and by disintegrins, naturally occurring small proteins that contain RGD-like sequences and inhibit ligand binding in the same way as RGD peptides.

GPIIb and GPIIIa are highly polymorphic. These polymorphisms are important in platelet transfusion reactions, causing neonatal alloimmune thrombocytopenic purpura (NITP) in infants and post-transfusion purpura (PTP) in transfusion recipients. Recent studies suggest that there may be an association between the PI (Zw) allele on GPIIIa and unstable coronary artery syndromes. Persons positive for PI^{A1} have a leucine at position 33 of mature glycoprotein IIIa and have a lower risk of development of symptomatic coronary artery heart disease than persons positive for PI^{A2}, who have a proline at this position. It has been suggested that this polymorphism, which is the result of the substitution of cytosine for thymidine at position 1565 in exon 2 of the glycoprotein IIIa gene, affects the affinity of GPIIb/IIIa for fibrinogen.

Several antagonists of GPIIb/IIIa binding of fibrinogen have been developed for clinical use (**Table 1**). c7E3 Fab (abciximab; ReoPro) is the first GPIIb/IIIa antagonist to be introduced for clinical use. It is a Fab fragment of a murine/human chimeric monoclonal antibody to GPIIb/IIIa which binds to the ligand binding site and blocks fibrinogen binding. It recognizes both GPIIb/IIIa and the vitronectin receptor ($\alpha v\beta 3$) and has a high affinity for the receptor and low off rate. c7E3 Fab has been engineered to minimize the likelihood of an immune reaction by substituting human constant regions for the murine constant regions. Nevertheless, in the EPIC trial, there was a 6.5% rate of antibody formation to the murine component of c7E3. Integrilin is a peptide GPIIb/IIIa antagonist which is based

Table 1 Characteristics of GPIIb/IIIa Antagonists

Agent	Class	Size	Stoichiometry	T1/2
Abciximab	Fab mAb	~50,000	1.5:1	6-12 h
Eptifibatide	cyclic peptide	~800	100:1	2-3 h
Tirofiban	nonpeptide mimetic	~500	100:1	2-3 h
Lamifiban	nonpeptide mimetic	~500	100:1	2-3 h
Xemilofiban	nonpeptide mimetic	~500		4 h
Sibrafiban	nonpeptide mimetic	~500		2.3 h
Lefradafiban	nonpeptide mimetic	~500		

on the sequence of Barbourin, a disintegrin isolated from the venom of the southeastern pygmy rattlesnake, *Sistrurus m. barbouri*. Barbourin was demonstrated to be a specific inhibitor of GPIIb/IIIa, which was specific by virtue of a KGD sequence, instead of an RGD sequence, which interacts with the ligand binding site of GPIIb/IIIa. Integrilin is a cyclic heptapeptide that has high affinity and is specific for GPIIb/IIIa. It has a short plasma half-life and low immunogenicity. Tirofiban (Aggrastat, MK-383) and Lamifiban (Ro-44-9883) are optimized non-peptide compounds synthesized on a tyrosine template, which mimic the charge and spatial orientation of RGD-containing ligands. Like the peptide inhibitors, these non-peptide mimetics are small, have a relatively short half-life, and are non-immunogenic. Oral GPIIb/IIIa antagonists, including Xemilofiban (SC-54684A), Sibrafiban (Ro-48-3657), Lefradafiban (BIBU-104), and Orbofiban (SC-57099B), are orally active prodrugs that are metabolized into the active compound. They are structurally similar to the parenteral non-peptide mimetics and have half-lives that are affected by metabolism and clearance.

GPIIb/IIIa antagonists have been examined in a variety of coronary syndromes and are licensed for clinical use. Large, placebo-controlled, randomized clinical trials have been performed to examine the effect of GPIIb/IIIa antagonists in the setting of percutaneous coronary intervention (EPIC, EPILOG, Simoons et al, CAPTURE, RAPPORT, IMPACT, IMPACT-II, Kereiakes et al, and RESTORE) and non-ST segment elevation acute coronary syndromes (Schulman et al, PURSUIT, PRISM, PRISM-PLUS, Theroux et al, and PARAGON). In a meta-analysis of percutaneous coronary intervention trials, for the combined end-point of death and non-fatal myocardial infarction and for the composite end-point of death, myocardial infarction, or revascularization, at 48-96 hours, at 30 days, and at six months, there is a significant benefit favoring GPIIb/IIIa antagonists. No significant difference in mortality was observed. Overall, for the composite end-point of death, myocardial infarction, or revascularization, at six months there were 23 fewer events per 1000 patients treated. In a similar meta-analysis of acute coronary syndrome trials, for the combined end-point of death or non-fatal myocardial infarction and for the composite end-point of death, myocardial infarction, or revascularization, at 48-96 hours and at 30 days, there is a significant benefit of the GPIIb/IIIa antagonists. For the composite end-point of death, myocardial infarction, or revascularization, this statistical significance also extends to six months but not for the combined end-point of death or non-fatal myocardial infarction. Again, there was no significant difference in mortality at any time point. The overall benefit at six months for the composite end-point of death, myocardial infarction, or revascularization was 20 fewer events per 1000 patients treated.

Glycoproteins Ia and IIa (VLA-2, $\alpha 2\beta 1$)

GPIa/IIa is a receptor for collagens type I and IV and mediates von Willebrand factor independent platelet adhesion

to the vessel wall. Like GPIIb/IIIa, GPIa and GPIIa are member of the integrin family of adhesion receptors. Binding of collagen by GPIa/IIa is thought to be mediated by a GPAGKGDGEAGA sequence present in the $\alpha 1(I)$ -CB3 peptide of collagen. The integrin sequences that mediate the interaction with collagen reside in a broad sequence called the I domain in the extracellular portion of the molecule. Unlike GPIIb/IIIa, GPIa/IIa is constitutively active and does not require activation to interact with collagen.

Deficiency of GPIa/IIa results in a mild bleeding disorder characterized by an abnormal platelet response to collagen. Increases in the number of GPIa/IIa receptors on the platelet surface may also affect platelet function. Two alleles of the GPIa gene, designated C807 and T807, appear to be associated with low or high platelet GPIa/IIa density, respectively, and with slower or faster rate of platelet adhesion to type I collagen. Two studies support the clinical importance of the density of GPIa/IIa on the platelet surface. Evidence has been presented that the density of GPIa/IIa can moderate the bleeding tendency in von Willebrand's disease such that a high GPIa/IIa density on the platelet surface can reduce the bleeding tendency in von Willebrand's disease while a low density can increase the bleeding tendency. Conversely, there is reported to be an association between the T807 allele with the attendant high receptor density and nonfatal MI among individuals younger than the mean age of 62 years. Although the mechanism of this association is not proven, the implication is that increased platelet adhesion to collagen increases the risk of platelet mediated thrombosis after plaque rupture.

Antagonists to GPIa/IIa have not been developed for clinical use, but the preceding suggests that such agents might be useful for modifying platelet behavior. Platelet adhesion to collagen is the initial event in vascular responses to injury and is probably the initial event in coronary thrombosis. Inhibition of this interaction might be expected to be an effective mechanism for reducing the complications of coronary atherosclerosis.

Glycoproteins Ib, IX, and V

The GPIb, which consists of two disulfide-linked subunits (GPIb α and GPIb β) and GPIX are members of the leucine-rich glycoprotein (LRG) gene family. This family of proteins is characterized by a common structural motif in the extracellular domain composed of a leucine-rich sequence, PXXLLXXXXLXXLXLSXNXLXXL. GPIb α contains seven leucine-rich repeats in the extracellular domain, while GPIb β and GPIX each contain a single leucine-rich repeat in the extracellular domain. GPIb and GPIX form a tight complex on the surface of platelets and associate with another platelet membrane glycoprotein, GPV, with a stoichiometry of 2:2:1. Complex formation between GPIb and GPIX, but not GPV, is required for surface expression.

The GPIb/IX complex mediates the von Willebrand factor-dependent adhesion of platelets to collagen. Adhesion is increased by shear, which is thought to induce a structural change in the receptor that enhances the interaction

with von Willebrand factor. The A1 domain of von Willebrand factor forms the principal binding site for GPIb and has been recently crystallized, yielding new information about putative sequences involved in the interaction with GPIb. The A3 domain of von Willebrand factor mediates the interaction with collagens type I and III and has also been recently crystallized. The model derived from the crystal structure suggests that the von Willebrand factor-collagen interaction is primarily through charge interactions between negatively charged residues in the von Willebrand factor A3 domain and positively charged residues in collagen. The site of von Willebrand factor binding in GPIb is less well defined. Binding is thought to occur in the amino-terminal 45 kDa tryptic fragment. Within this region of GPIb, an anionic site, YDYYPEE²⁸², containing two sulfated tyrosine residues, at tyrosine 278 and tyrosine 279, has been further implicated in von Willebrand factor binding. Inhibitory monoclonal antibodies to GPIb and peptide antagonists have been described.

Two linked genetic polymorphisms have been reported in the coding sequence of the gene encoding the α -chain of GPIb: a C/T transition at nucleotide 1018 that results in athreonine/methionine dimorphism at amino acid 145 (Kob/a alloantigen system) in the vWF-binding domain of the receptor, and a variable number of 13-amino acid sequence tandem repeats (VNTRs) in the macroglycopeptide region of the GPIb α gene. Threonine at amino acid 145 is linked with alleles expressing one or two VNTRs, whereas methionine at amino acid 145 is linked with alleles expressing three or four VNTRs. Epidemiological studies suggest that the presence of the methionine/3-4 VNTR allele in GPIb α is a risk factor for the prevalence and severity of coronary artery and cerebrovascular diseases but not venous thromboembolism. No functional differences in the polymorphic forms of GPIb that might account for the increased risk of coronary artery and cerebrovascular diseases have been identified.

As much or more than GPIa/IIa, GPIb/IX is an attractive target for platelet inhibition because of the relatively large number of receptors on the platelet surface and its prominent role in the initial platelet responses to vascular injury. Snake venom inhibitors of GPIb/IX interaction with von Willebrand factor may provide initial approaches to regulation of this interaction.

Glycoproteins Ic and IIa (VLA-5, $\alpha 5\beta 1$)

GPIc/IIa is a ubiquitously expressed receptor for fibronectin which is present on the platelet surface and mediates the interaction of platelets with fibronectin. GPIc and GPIIa are members of the integrin superfamily. GPIIa ($\beta 1$) is able to form a heterodimer complex with either GPIa to generate a collagen receptor, with GPIc for generate a fibronectin receptor, or with GPIc' to generate a laminin receptor as indicated below. Like GPIa/IIa, GPIc/IIa is constitutively active and binds fibronectin without requiring platelet activation. There are two sequences in fibronectin which inter-

act with GPIc/IIa: an RGD sequence in the tenth type III repeat which interacts primarily with the $\beta 1$ subunit and a synergy sequence in the adjacent ninth type III repeat which interacts primarily with the $\alpha 5$ subunit.

Vitronectin receptor ($\alpha v\beta 3$)

Platelets express small numbers of the vitronectin receptor on their surface. The vitronectin receptor, like GPIIb/IIIa, is activatable. The presence or absence of the vitronectin receptor on the surface of platelets from patients with Glanzmann's thrombasthenia has been used to assign the molecular defect to either GPIIb or GPIIIa. Thus, if there is no detectable vitronectin receptor, it implies that the molecular defect responsible for the Glanzmann's thrombasthenia resides in the GPIIIa molecule.

Endothelial cell $\alpha v\beta 3$ has been shown to play an important role in angiogenesis and is currently the subject of intense interest as a target for anti-cancer approaches. $\alpha v\beta 3$ has been shown to mediate the angiogenic effects of basic fibroblast growth factor (bFGF) and tumor necrosis factor alpha (TNF α) and inhibition of $\alpha v\beta 3$ inhibits the angiogenic responses to these agents but not to vascular endothelial growth factor (VEGF). In a variety of animal models, inhibition of $\alpha v\beta 3$ inhibits tumor cell growth and metastasis by inhibiting vascularization of the tumor. Clinical trials of $\alpha v\beta 3$ antagonists in cancer are currently underway.

Glycoproteins Ic' and IIa (VLA-6, $\alpha 6\beta 1$)

GPIc'/IIa constitutes the laminin receptor on the platelet surface. Immunoprecipitation studies suggest that GPIc'/IIa may exist on the cell surface in a complex with proteins with four transmembrane domains, so-called TM4 proteins, such as CD9, CD81, and the novel TM4 protein NAG-2. GPIc'/IIa recognizes a sequence in the long-arm E8 fragment obtained after elastin digestion of laminin. The binding requires the presence of divalent cations that bind to specific sites on the integrin alpha subunit. Recent studies suggest that the laminin receptor may play a role in megakaryocyte differentiation.

Glycoprotein IV (CD36)

CD36 is a highly glycosylated transmembrane protein present on platelets, monocytes, endothelial cells, and nucleated erythrocytes. CD36 is a receptor for thrombospondin and for collagen. The thrombospondin binding site has been mapped to a single disulfide loop in the extracellular domain of GPIV but the collagen binding site is unknown.

GPIV is constitutively phosphorylated in platelets. Evidence has been presented to indicate that the binding of collagen and thrombospondin by GPIV appears to be regulated by phosphorylation. Dephosphorylation of GPIV favors binding of thrombospondin and a reciprocal decrease in collagen binding, while phosphorylation favors binding of collagen and decreased thrombospondin binding. In resting platelets, CD36 exists in a complex with src-related protein tyrosine kinases, including lyn, fyn, yes, and chk/hyl.

Conclusion

In summary, the platelet surface is a highly complex interfaces with blood and the vessel wall. Proteins on the platelet surface mediate the interaction of platelets with vessel wall components and other cells. Increased understanding of the molecular mechanisms involved in the interaction of these proteins with their ligands has led to the development of new concepts for regulating platelet function. At present,

antagonists of GPIIb/IIIa are in clinical trials and in the clinic and show benefit in a variety of coronary syndromes. Orally active antagonists of GPIIb/IIIa promise to provide new approaches to the long-term treatment of vascular disorders. Based on the initial success of the GPIIb/IIIa antagonists, it is anticipated that inhibitors of other platelet membrane glycoproteins might be developed and provide additional ways of modifying platelet function.