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Review

Glycoprotein IIb/IIIa antagonists – from bench to practice

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Abstract. The central role played by the $\alpha_{IIb}\beta_3$ receptor in platelet aggregation, and hence in platelet thrombosis, has led to the development of a number of parenteral and oral glycoprotein (GP) IIb/IIIa inhibitors for use in cardiovascular disease states, such as acute coronary syndromes and stroke. The predominant effect of these agents is to inhibit platelet aggregation, although studies of $\alpha_{IIb}\beta_3$ receptor function and various GP IIb/IIIa inhibitors have demonstrated the potential for these agents to produce effects on other aspects of platelet function, in addition to non-platelet effects. Overall, clinical studies have demonstrated an impressive beneficial effect for parenteral agents in reducing ischemic complications following percutaneous intervention, and a more modest beneficial effect in the treatment of patients with acute coronary syndromes. Trials with oral GP IIb/IIIa inhibitors in similar patient populations have demonstrated toxicity, manifested by an increased mortality in treated patients. Increased understanding of molecular aspects of both $\alpha_{IIb}\beta_3$ receptor function and the effects of GP IIb/IIIa inhibition may help explain some of the inconsistency in recently reported clinical studies with parenteral agents, and the frank toxicity of oral agents. Such studies may also hold the key to the development of newer agents with enhanced therapeutic benefit.

Key words. Platelet; thrombosis; $\alpha_{IIb}\beta_3$ receptor; glycoprotein IIb/IIIa inhibitor; acute coronary syndrome; myocardial infarction; percutaneous intervention.

Introduction

Platelets constitute an essential component of primary hemostasis by forming platelet thrombi at sites of vascular injury. Investigation over the last three decades, however, has demonstrated the pathological role of platelet thrombus formation in a number of cardiovascular disease states [1, 2] including acute coronary syndromes (ACS), transient ischemic attacks, and stroke. Platelet thrombosis has also been recognized to cause abrupt vessel closure following coronary arterial injury during percutaneous coronary intervention (PCI), especially during stenting [3, 4].

The pivotal role of the $\alpha_{IIb}\beta_3$ receptor in platelet thrombus formation was recognized from the study of patients with the autosomal recessive disorder, Glanzmann's thrombasthenia. Platelets in these patients fail to aggregate in response to all known platelet agonists [5]. In the early 1970s, two glycoproteins (GPs), GPIIb and GPIIIa, were observed to be absent from the platelets of these patients [6, 7]. Kunicki et al. [8] subsequently demonstrated that these two glycoproteins formed a Ca^{2+}-dependent complex on the platelet membrane. The receptor thus became a target for antithrombotic therapy.

The subsequent development of inhibitors of the $\alpha_{IIb}\beta_3$ receptor (termed GP IIb/IIIa antagonists), and their clinical application, represents a major achievement by both basic scientists and clinical investigators. Today, more

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than 100,000 patients with ischemic heart disease have been enrolled in clinical trials using these agents. Three parenteral GP IIb/IIIa blockers are approved for use by the US Food and Drug administration, with many more undergoing phase II and phase III trials for an increasing list of indications.

The goal of this article is to review our current understanding of GP IIb/IIIa antagonists, with respect to both their molecular mechanisms of action and clinical efficacy. A necessary pre-requisite for such a discussion is a review of the structure, regulation, and function of the $\alpha_{IIb}\beta_3$ receptor.

The $\alpha_{IIb}\beta_3$ receptor

Structure

The $\alpha_{IIb}\beta_3$ receptor (fig. 1) belongs to the integrin family of adhesion receptors [9] and is largely restricted to the surface of platelets and megakaryocytes [10]. It represents the most abundant protein on the platelet surface with ~80,000 copies per platelet in the resting state. There are an additional 40,000 copies of this receptor on the α-granule membrane within each platelet. Structurally, it resembles all integrin receptors in that it is composed of a non-covalently linked $\alpha\beta$ heterodimer. Each subunit is coded for by separate genes, ~1 Mb apart, on the long arm of chromosome 17q21.32 [11]. Both subunits have large extracellular domains containing the amino terminus, single transmembrane domains, and short cytoplasmic tails containing the carboxyl terminus (fig. 1). The larger α_{IIb} subunit is composed of a heavy chain (105 kDa) and light chain (25 kDa), formed following post-translational cleavage of the precursor polypeptide. There are four EF hands in the extracellular portion of the α_{IIb} subunit, which resemble the structural motif of the calcium-binding sites typified by helices E and F of parvalbumin. Molecular modeling of the amino terminal of the α_{IIb} peptide predicts a seven-bladed β propeller configuration with each blade of the propeller being formed by a repeat sequence [12]. The β_3 subunit is a single polypeptide of 762 amino acids, which contains 28 disulfide linkages. There are two highly conserved regions in this peptide subunit. The first extends from residues 110 to 294 near the amino terminus. This region contains a structural motif first identified in the α subunits of integrins called the inserted or I domain, within which there is a MIDAS domain (metal ion-dependent adhesion site) [13]. The second is a cysteine-rich repeat region (residues 423–622) containing 31 of the 56 cysteine residues in the subunit [14]. Following processing of the individual peptide subunits in the endoplasmic reticulum, heterodimerization occurs with 1:1 stoichiometry, and with a weak K_d in the micromolar range [15]. Divalent cations (principally Ca^{2+}), which have five potential binding sites on the $\alpha_{IIb}\beta_3$ receptor (i.e., four on the EF hand α_{IIb} subunit, and one on the MIDAS region on the β_3 subunit), serve to stabilize the complex by decreasing the dissociation rate. The receptor is subsequently expressed on the platelet surface. Electron micrographs of the assembled receptor demonstrate a globular head region, which lies extracellularly and contains the ligand-binding region, and two tails which form the transmembrane and intracellular portions of the receptor [16].

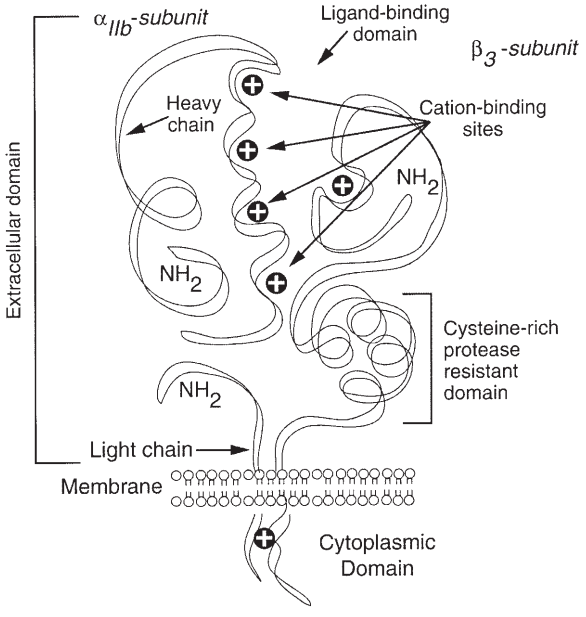

Figure 1. Schematic illustration of integrin $\alpha_{IIb}\beta_3$. Reproduced with permission from Topol et al. [73].

The ligand binding pocket in the extracellular domain of the receptor is formed by the amino-terminal components of both subunits. One of these is an Arg-Gly-Asp (RGD) consensus sequence-binding site in the vicinity of residues 109–171 on the β_3 subunit. Since roughly half of all integrins have such a binding site, additional sites in the immediate vicinity of this binding site confer the specificity of ligand binding for the $\alpha_{IIb}\beta_3$ receptor. These include a region extending from residues 145 to 224 on the α_{IIb} subunit [17]. Despite a span of 80 amino acids in the linear sequence of the α_{IIb} subunit, the critical residues involved form the upper surface of a single quadrant (involving the W2, W3, and W4 blades) of the proposed β propeller structure, at the amino terminus of the α_{IIb} subunit. An additional site at residues 164–202 on the β_3 subunit has been implicated in ligand binding [18].

The cytoplasmic tails of both subunits are important in transmembrane signaling across the receptor. While they lack any enzymatic activity, they provide anchor sites for the recruitment of signaling proteins to the membrane surface and for attachment of cytoskeletal proteins (e.g.,

actin, myosin [19], α-actinin [20], talin [21], and skemelin [22]) that serve to link the platelet actin cytoskeleton to the $\alpha_{IIb}\beta_3$ receptor. These cytoskeletal attachments play a critical role in many aspects of $\alpha_{IIb}\beta_3$ receptor function.

Regulation of $\alpha_{IIb}\beta_3$ receptor activation state

Under normal hemostatic conditions, platelets do not interact with the vessel wall or with one another. The endothelium plays an important role in preventing such interactions by forming a physical barrier between circulating blood components and the subendothelial matrix, and perhaps more importantly by secreting such components as prostacyclin (PGI_2), nitric oxide (NO), and ADPase which prevent platelet activation. Vessel wall injury, which occurs as a result of spontaneous atherosclerotic plaque rupture or fissuring in ACS, or intentional plaque disruption during PCI, results in loss of the non-thrombogenic properties of the endothelium and exposure of blood components to the subendothelial matrix. The principal interaction mediating initial platelet contact is between binding sites on the A1 domain of von Willebrand factor (vWF) and the GP 1b receptor, which exists in a complex with GP IX and GP V [23]. Subsequently, a number of integrin and non-integrin receptors on the platelet surface mediate platelet adhesion to subendothelial matrix proteins such as collagen, fibronectin, laminin, and vWF. Platelet activation ensues from the combined interaction of platelets with these adhesive proteins and with soluble agonists, such as thrombin, ADP, and adrenaline, which accumulate in the surrounding microenvironment.

Platelet activation has a number of phenotypic features. There is a dramatic alteration in platelet shape from a disc-shaped cell fragment to a spiculated sphere with narrow finger-like extensions, termed filopodia, and broader intervening extensions, termed lamellipodia [24, 25]. Filopodia are associated with the accumulation of bundles of long actin filaments, while lamellipodia are associated with rearrangement of the platelet actin skeleton into orthogonal arrays of short filament networks.

Platelet secretory granules (dense core and α granules, and lysosomes) become centralized and fuse with invaginations in the plasma membrane, termed the open canalicular system [26]. Their contents are subsequently discharged and exert a multitude of actions including enhanced platelet activation (ADP, serotonin) and blood coagulation (heparin antagonist/platelet factor 4, fibrinogen, factor Va), inhibition of fibrinolysis (plasminogen activator inhibitor-1), and promotion of cell growth/proliferation [platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β]. Granule secretion is associated with an increase in intracellular Ca^{2+} levels and cytoskeletal rearrangements.

Finally, platelet aggregation occurs as a result of $\alpha_{IIb}\beta_3$ receptor activation. Activation of this receptor allows binding of soluble fibrinogen. The terminal-chain dodecapeptide sequence appears to be the critical region of the fibrinogen macromolecule that mediates attachment to the $\alpha_{IIb}\beta_3$ receptor-binding pocket. Because fibrinogen is bivalent, it promotes platelet-platelet interaction and thrombus formation. Other ligands such as vitronectin, fibronectin, and thrombospondin may bind the activated $\alpha_{IIb}\beta_3$ receptor. While the concentration of these ligands in plasma is low, suggesting they play a minor role compared to fibrinogen, the release of thrombospondin from activated platelets may increase the concentration locally such that it may become a significant ligand. It is important to note that all platelet agonists mediate their effects on platelet aggregation through $\alpha_{IIb}\beta_3$ receptor activation.

Platelet aggregation is the most significant feature of platelet activation, since aggregation determines the cellular mass of the thrombus and hence its pathological consequence in disease states. Given the pivotal role of the $\alpha_{IIb}\beta_3$ receptor in this process, it is not surprising that the activation state of this receptor is carefully regulated in order to prevent inadvertent platelet aggregation. Platelet agonists and antagonists regulate the receptor by a process known as inside-out signaling, whereby the ligand-binding affinity of the receptor is modulated by signals generated within the cell. The major pathways involved in inside-out signaling are described in the following section (fig. 2).

Inhibitors of $\alpha_{IIb}\beta_3$ receptor activation

As alluded to earlier, prostaglandins (PG) I_2 and E_2 and nitric oxide serve to maintain the platelet and $\alpha_{IIb}\beta_3$ receptor in a quiescent state. In this state, the receptor only allows activation-independent ligand binding of immobilized fibrinogen and prothrombin. These platelet antagonists are produced by the endothelium (PGI_2, NO) and by the platelet itself (PGI_2, PGE_2, NO). PG I_2 and PG E_2 bind to IP and EP (predominantly EP4) receptors on the platelet surface, which are coupled to the Gs family of G proteins [27]. Activation of the α subunit of the Gs protein results in elevated cyclic AMP levels within the platelet. Nitric oxide acts by diffusing through the platelet membrane and interacts with the heme group of soluble guanylate cyclase, inducing a conformational change in the enzyme, and stimulating the conversion of GTP to cyclic GMP [28]. Elevated levels of the cyclic nucleotides, cAMP and cGMP, have been demonstrated to inhibit platelet aggregation. Their effects on platelet aggregation are mediated through stimulation of their respective cAMP- and cGMP-dependent protein kinases, PKA and PKG. These kinases may affect the activity of the $\alpha_{IIb}\beta_3$ receptor by modulating the activity of various enzymes demonstrated to play a role in platelet agonist mediated $\alpha_{IIb}\beta_3$ receptor activation [e.g., phospholipase A2, phospholipase C, phosphatidylinositol (PI) 3-kinase]

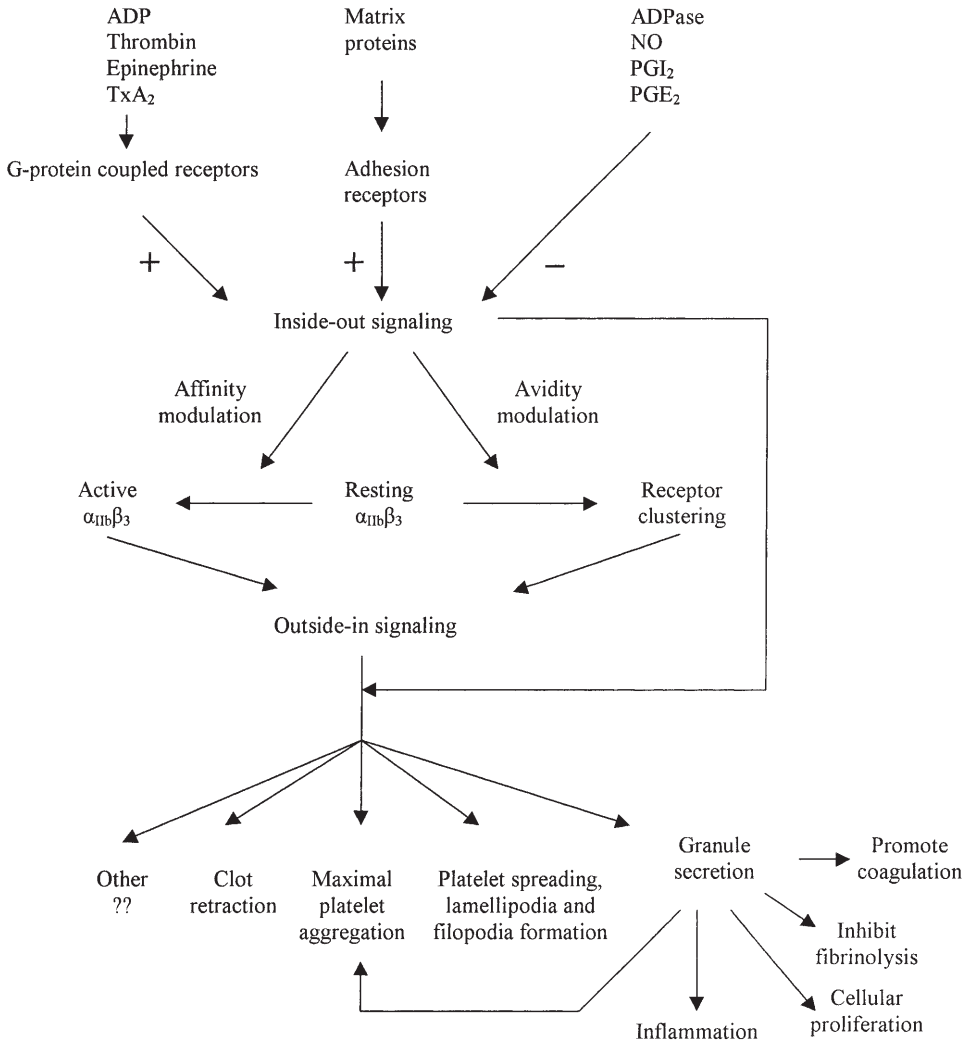

Figure 2. Schematic illustration of $\alpha_{IIb}\beta_3$ receptor regulation: various inhibitors and promoters of $\alpha_{IIb}\beta_3$ receptor activation act on inside-out signaling pathways to regulate the flux between the activated and resting states of the $\alpha_{IIb}\beta_3$ receptor. Outside-in signaling pathways activated by $\alpha_{IIb}\beta_3$ receptor activation act in concert with inside-out signaling pathways to effect a number of platelet and non-platelet effects.

[29], or by directly phosphorylating the receptors of platelet agonists [30, 31]. Less well recognized potential agents that inhibit $\alpha_{IIb}\beta_3$ receptor activation include CD39 and PDGF. CD39, an ecto-ADPase, is a constitutively expressed enzyme that is attached to the platelet membrane and blocks the aggregation response by metabolizing ADP [32]. A role for PDGF in regulating platelet aggregation is suggested by an experiment which demonstrated that pre-incubation of platelets with PDGF inhibited thrombin-induced platelet aggregation [33]. This action of PDGF is potentially mediated by autocrine stimulation of PDGFα receptors on the platelet surface.

Promoters of $\alpha_{IIb}\beta_3$ receptor activation

Table 1 provides a list of the most important platelet agonists, their receptors, and principal signaling pathways in platelets. These pathways undoubtedly generate an element of frustration and confusion to readers hoping to make sense of the myriad of receptors, second messengers, and potential effectors. Adding to the confusion is the inherent redundancy in the process of platelet activation. Many agonists have multiple receptors. Each receptor generates multiple signals with multiple potential effectors. There is considerable cross-talk between pathways activated by different agonists. In addition, although there are reasonable candidates, the terminal effector molecules directly effecting $\alpha_{IIb}\beta_3$ receptor activation are unknown. Finally, the precise events at the level of the $\alpha_{IIb}\beta_3$ receptor itself which result in $\alpha_{IIb}\beta_3$ receptor activation are not known with certainty. Rather than attempt to describe all of these pathways, the signal pathways of thrombin, one of the most potent platelet agonists are il-

Table 1. List of platelet agonists, their receptors, and signal transduction pathways.

Agonist	Receptor	Signal transduction pathway
Adenosine diphosphate (ADP)	$P2Y_{ADP}$ ($P2T_{PLC}/P2_{Y1}$) $P2Y_{12}$ P2X1	Gq-coupled → activation of PLC_β Gi-coupled → inhibition of adenylate cyclase ligand-gated ion channel, increase intracellular Ca^{2+}
Thrombin	PAR 1 PAR 4	Gi → inhibition of adenylyl cyclase, activation of non-receptor tyrosine kinases, G protein coupled receptor kinases, PLC_β, PI3K, K^+channel Gq → activation of PLC_β $G_{12/13}$-coupled → activation of Rho proteins
Epinephrine	α_2	Gi-coupled → inhibition of adenylyl cyclase, activation of PLC
Thromboxane	TPα TPβ	$Gq/G_{11}/G_{16}$-coupled → activation of PLC_β Gi-coupled → activation of Src G_{12}/G_{13}-coupled → activation of PLC_β, PLA_2, Rho/Ras family proteins G_h-coupled → activation PLC_δ
Collagen	$\alpha_2\beta_1$ GP VI	tyrosine phosphorylation and activation of Syk, phosphorylation of $PLC_{\gamma 2}$ phosphorylation of Fcγ chain protein, recruitment and activation of Syk
VWF	GP Ib/IX/V complex	association with 14-3-3ζ association with tyrosine kinases and activation of Syk
Immobilized fibrinogen	GP IIb/IIIa	Activation of tyrosine kinases, activation of PLC, PI3K, SHIP

Reproduced with modifications from Law et al. [45] with permission. PLC, phospholipase C; PI3K, phosphoinositide-3 kinase; PLA_2, phospholipase A_2; GP, glycoprotein; SHIP, SH_2-containing inositol-5-phosphatase.

lustrated in figure 3 [34, 35]. These pathways serve to illustrate the caveats highlighted above and to demonstrate the major players common to many of the signal pathways transducing the signal from platelet agonist receptor binding to the $\alpha_{IIb}\beta_3$ receptor.

Mechanism of $\alpha_{IIb}\beta_3$ receptor activation

Our current understanding is that $\alpha_{IIb}\beta_3$ receptor activation is caused by two processes termed affinity and avidity modulation [36, 37]. Both processes serve to enhance ligand binding to the $\alpha_{IIb}\beta_3$ receptor, although affinity modulation is generally believed to be the predominant mechanism. Affinity modulation is assumed to result from conformational change in the receptor, since no chemical modification of the $\alpha_{IIb}\beta_3$ receptor during activation has been documented. Fluorescence energy transfer experiments that have detected the relative movement of labeled antibodies bound to the two subunits of the receptor during activation are consistent with this assumption [38]. The conformational change in the receptor results in exposure of the fibrinogen-binding site such that it can interact with circulating fibrinogen. Studies of naturally occurring and experimentally induced integrin mutations have demonstrated a critical role for the cytoplasmic tails of both the α_{IIb} and β_3 subunits in generating the conformational change in the receptor in response to inside-out signaling [31, 39]. Regulatory proteins are believed to interact with the membrane-distal tail residues, inducing an alteration in a hinge-like region in the membrane-proximal portion of the α_{IIb} and β_3 cytoplasmic tails, which in turn propagates across the membrane to induce a conformational change in the extracellular portion of the receptor [37]. Such regulatory proteins may include intracellular proteins (e.g., β_3 endonexin, calcium and integrin binding protein/CIB) or transmembrane proteins (e.g., CD47, CD98, tetraspanins) that directly or indirectly bind to the $\alpha_{IIb}\beta_3$ receptor.

Avidity modulation of the $\alpha_{IIb}\beta_3$ receptor by platelet agonists is mediated by the clustering of $\alpha_{IIb}\beta_3$ heterodimers into oligomers. In the resting state, the $\alpha_{IIb}\beta_3$ receptor is apparently constrained by either direct or indirect attachments to the cytoskeleton [19–22]. Effector molecules, such as the Ras and Rho family GTPases, generated by signal pathways activated by platelet agonists, interact with proteins that regulate actin assembly [25, 40]. These include such proteins as barbed-end capping proteins (e.g., gelsolin) and disassembly proteins (e.g., cofilin). Subsequent cytoskeletal rearrangements relieve the constraint on the $\alpha_{IIb}\beta_3$ receptor enabling lateral diffusion of $\alpha_{IIb}\beta_3$ receptors and development of oligomers [41]. This process occurs after the conformational change in the $\alpha_{IIb}\beta_3$ receptor and mediates irreversible fibrinogen binding.

$\alpha_{IIb}\beta_3$ receptor function

Platelet effects

Following ligand attachment to the $\alpha_{2b}\beta_3$ receptor, a number of post-ligand binding events occur. A large number of intracellular signals are generated by a process known as outside-in signaling. These events, and the molecular processes mediating them, are important

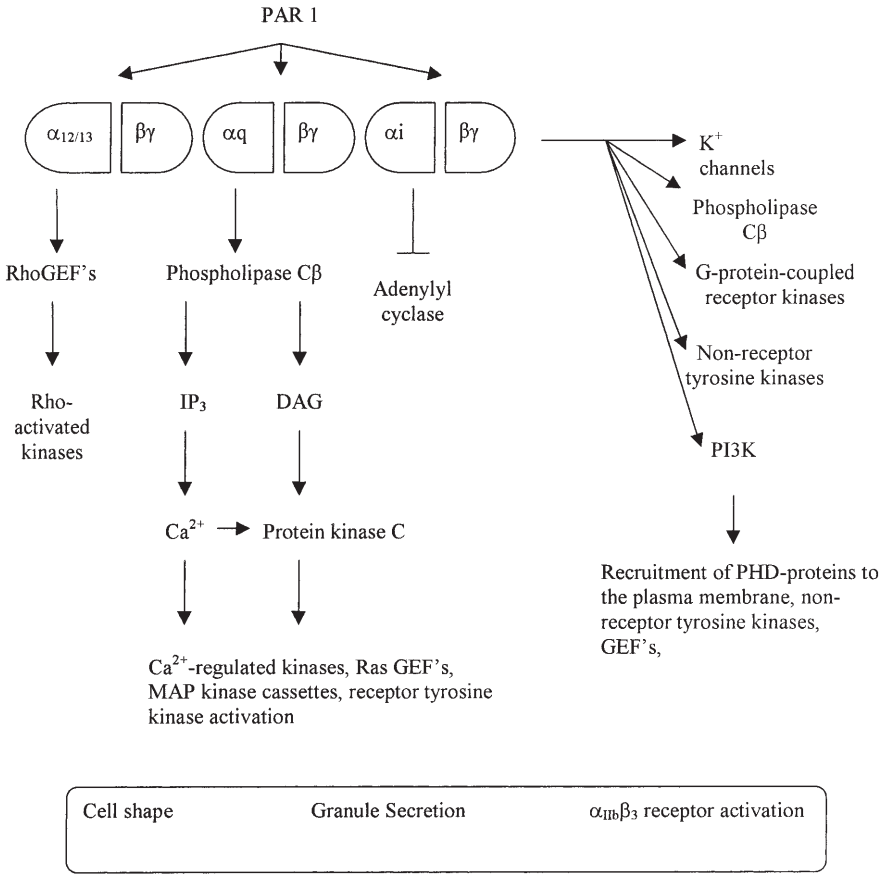

Figure 3. Signaling pathways activated by thrombin. The PAR-1 thrombin receptor is coupled to members of the $G_{12/13}$, G_q, and G_i families of G proteins. The α subunit of each of these proteins and the $\beta\gamma$ subunit of G_i protein activate multiple signaling pathways contributing to $\alpha_{IIb}\beta_3$ receptor activation (i.e., inside-out signaling) and cytoskeletal effects resulting in alteration in platelet shape and granule secretion. GEF, guanine-nucleotide exchange factor; DAG, diacylglycerol; IP3, inositol triphosphate; MAP, mitogen-activated protein; PAR, protease activated receptor; PHD, pleckstrin homology domain; PI3K, phosphoinositide-3 kinase. Reproduced with permission from Coughlin et al. [35].

to the discussion of GPIIb/IIIa inhibitors. The therapeutic and adverse effects of these agents are determined not only by their ability to block the binding of fibrinogen to the extracellular portion of the receptor, but also by their ability (or failure) to block the myriad of other events triggered by outside-in signaling across the receptor. Outside-in signaling results in further platelet spreading, granule secretion, and clustering of the $\alpha_{IIb}\beta_3$ receptor (fig. 2). These effects serve to further activate $\alpha_{IIb}\beta_3$ receptors and maximize platelet aggregation. In addition, outside-in signaling promotes fibrin clot retraction, which results in clot stabilization. Many of the post-ligand binding events are mediated through effects on the platelet cytoskeleton. However, events other than cytoskeletal reorganization are also modulated by outside-in signaling [42].

The generation of integrin multimer complexes by platelet agonists, together with ligand-binding appears critical in generating outside-in signaling. Co-clustering of ligand-occupied integrin, integrin-associated adapters, enzymes, substrates, and cytoskeletal protein within complexes, termed focal complexes, generates the signals that mediate the effect of integrin binding on cytoskeletal reorganization and other platelet processes. Larger focal complexes, termed focal adhesions, subsequently become the sites of signal generation. Many of the details of the molecular and biochemical mechanisms mediating outside-in signaling are beginning to be elucidated. However, the precise functional consequences of these signaling pathways in human platelets is more speculative.

Several waves of tyrosine phosphorylation occur during outside-in signaling [37, 43]. Each of these waves of phosphorylation appears to be associated with distinct morphologic platelet effects. The Syk and $pp60^{src}$ protein tyrosine kinases are phosphorylated within seconds of ligand binding, independent of actin polymerization. Recently, Syk has been demonstrated to activate an adapter molecule SLP-76, which relays signals to several effectors involved in actin polymerization (e.g., Vav, Nck, SLAP-130) [44].

The other significant phosphorylation reactions that have been described appear dependent on initial polymerization of actin molecules. These include phosphorylation of the integrin cytoplasmic domain (ICY) of the β_3 subunit and of a variety of tyrosine kinases (e.g., FAK). Several lines of evidence underlie the importance of the ICY domain of the β_3 subunit [45]. This region contains two NXXY motifs, 11 amino acids apart. The first extends from residues 744 to 747 and forms a tight β turn, while the other extends from residues 756–759 and lies within a β sheet structure. Platelet aggregation is necessary and sufficient for phosphorylation of both tyrosine residues [19]. Tyrosine phosphorylation of the β_3 cytoplasmic tail may mediate effects through a number of mechanisms. It may result in the physical linkage of the $\alpha_{IIb}\beta_3$ receptor to the cytoskeleton. This may occur through effects on the membrane-proximal region of the β_3 subunit, which has been demonstrated to bind cytoskeletal proteins [21, 22]. These proteins serve to link the receptor to the actin-myosin skeleton of the platelet. Direct cytoskeletal attachment between the tail domain of myosin heavy-chain protein and the phosphorylated site on the β_3 subunit has also been demonstrated [19]. These receptor cytoskeleton interactions may provide alignment for post-aggregation contractile events such as clot retraction. This phosphorylation also allows for the recruitment of signaling complexes to the membrane. Recently, the p52 isoform of the adaptor molecule Shc has been shown to bind directly to mono- and di-phosphorylated β_3 peptides in human platelets and to be the primary protein that associated in a phosphotyrosine-dependent manner with the β_3 peptide [46]. This protein is subsequently phosphorylated, and binds a further adapter molecule Grb2, which activates the Ras signaling pathway. The mitogen-activated protein kinase (MAPK) effectors of this pathway (Erk 1 and 2, p38) in turn activate phospholipase A_2, which generates arachidonic acid metabolites and stabilizes platelet-platelet aggregates. Other potential consequences of MAPK include effects on cell survival or protein transcription [42].

Phosphorylation of focal adhesion kinase (FAK) occurs only after full spreading and aggregation of platelets [43]. These enzymes represent one of the predominant proteins in focal adhesions. They are incapable of phosphorylating other substrates directly but may indirectly do so by binding to adapter/docking proteins such as paxillin, tensin, $p130^{cas}$, and Grb2/SOS [37]. These proteins in turn activate several downstream signaling molecules, including the small GTP-binding proteins of the Ras (e.g., raf) and Rho (e.g., Rho A, CDC42, Rac) families.

Following the wave of tyrosine phosphorylation, dephosphorylation occurs and appears to correlate with the process of fibrin clot retraction [47]. This dephosphorylation results from cytoskeletal recruitment and activation of tyrosine phosphatases. μ-Calpain, a Ca^{2+}dependent thiol protease, which is activated as a consequence of ligand-integrin binding, may also cause tyrosine dephosphorylation by cleaving several protein kinases such as PKC, $pp60^{c\text{-}src}$, and FAK [40]. Additional effects of μ-calpain may be mediated through proteolytic cleavage of a wide variety of targets including cytoskeletal proteins, signaling molecules, or the β_3 integrin unit. The effects of μ-calpain appear to be particularly important in lamellopodia formation.

An additional second messenger system that has been shown to play an important role in outside-in signaling is the phosphoinositide system. Engagement of the $\alpha_{IIb}\beta_3$ receptor results in relocation to the cytoskeleton of the p85/PI 3-K isoform of phosphoinositide 3-kinase, where it has been demonstrated to bind to the FAK, $p125^{FAK}$ [48]. The subsequent activation of PI 3-kinase generates D3 phosphoinositides, predominantly phosphoinositide 3,4 biphosphate (PI 3,4-P_2) and phosphoinositide 3,4,5 triphosphate (PI 3,4,5-P_3) [49]. The latter have been shown to mediate actin assembly and filopodial extension [50]. By blocking the interaction of capping proteins (e.g., gelsolin) with the barbed ends of actin filaments, these D3 phosphoinositides block actin filament fragmentation and promote actin polymerization and assembly. The activity of PI 3-kinase also appears important in maintaining the activated conformational state of the $\alpha_{IIb}\beta_3$ receptor following the onset of platelet aggregation and thus may be important in the production of stable platelet aggregates [51].

In addition to outside-in signaling, ligand binding to the $\alpha_{IIb}\beta_3$ receptor results in the formation of neoantigenic eptitopes [ligand-induced binding sites (LIBS)] on both the α_{IIb} and β_3 subunits by virtue of the conformational change in the receptor. Ligands may exert a differential effect on both the degree and spectrum of individual LIBS expression. For example, RGDS peptide exerts a significantly greater effect on expression of the D3 LIBS compared to H12 (terminal δ carboxy dodecapeptide of the gamma chain of fibrinogen). In addition, Cierniewski et al. [137] demonstrated that two cyclic peptides (cRGD and cHarGD) induced a distinct pattern of expression of the neoepitopes LIBS1 and PMI-1. The functional significance of neoepitope expression following binding of endogenous ligand is unknown. These neoepiptopes may have direct functional consequences or merely represent epiphenomena of conformational change in other portions of the receptor that influence receptor function.

Non-platelet effects

Activation of the $\alpha_{IIb}\beta_3$ receptor has important effects on extra-platelet events such as coagulation, inflammation and cell proliferation. This explains the potential for GP IIb/IIIa antagonists to have effects beyond inhibition of platelet aggregation.

Regarding blood coagulation, platelets have been demonstrated to have a number of potentially important effects. Activated platelets express specific receptors for factor Va and VIIIa, release an activated form of factor Va from α granules [52], and phosphatidyl serine and other anionic phospholipids that are usually localized in the inner leaflet of the platelet membrane become exposed on the surface [53]. These effects serve to promote the formation of the prothrombinase complex (factors X and V, Ca^{2+}, and anionic phospholipid) on the platelet membrane, which converts prothrombin to thrombin [54]. Prothrombinase complex assembly is enhanced by cytoskeletal events that are influenced by $\alpha_{IIb}\beta_3$ receptor activation and subsequent outside-in signaling. In addition, the $\alpha_{IIb}\beta_3$ receptor binds prothrombin itself in both the resting and activated states. This interaction facilitates the conversion of prothrombin to thrombin by activated factor X [55]. Finally, activated platelets express CD40 ligand (CD40L, also known as CD154), a transmembrane protein structurally related to the cytokine tumor necrosis factor (TNF)-α, on their surface following α granule secretion [56]. This ligand interacts with CD40 on the surface of endothelial cells and monocytes. This interaction has been shown to induce tissue factor (which initiates the extrinsic pathway of blood coagulation) in both cell types and to downregulate the expression of thrombomodulin in endothelial cells [57]. These effects serve to induce a procoagulant phenotype.

Inflammation may also be affected by $\alpha_{IIb}\beta_3$ receptor activation. Platelet activation recruits inflammatory cells to the site of vascular injury by mediating adhesion of leukocytes to the platelet membrane itself or to the adjacent endothelial surface. Interaction between CD40L on the surface of activated platelets and endothelial cell CD40 results in the secretion of chemokines [e.g., interleukin-8(IL-8), monocyte chemoattractant protein-1(MCP-1)] and expression of adhesion molecules (e.g., E-selectin, ICAM-1, and VCAM-1) by the endothelial cell [56]. IL-8 and MCP-1 are potent chemoattractants for polymorphonuclear leucocytes (PMNs) and monocytes, respectively. Adhesion molecules such as ICAM-1 are important in the direct or indirect attachment through a fibrinogen bridge of activated PMNs to the endothelium [58]. P-selectin, expressed on the surface of the platelet following activation, is felt to be responsible for the initial attachment and rolling of leukocytes via the P-selectin glycoprotein ligand-1 receptor (PSGL-1) to the platelet surface [59]. Arrest of leukocytes on the platelet surface is mediated by the bridging of fibrinogen, bound to the activated $\alpha_{IIb}\beta_3$ receptor on the platelet surface, to the activated $\alpha M\beta_2$ (Mac-1) integrin receptor on the leukocyte surface [60]. In vitro, binding of activated platelets to myeloid leukocytes results in production of pro-inflammatory cytokines, mitogens, reactive oxygen species, and increased surface expression of Mac-1. Platelet activation also exerts effects on vascular inflammation by modulating the activity of these recruited inflammatory cells through its effects on the production of inflammatory mediators such as factor Xa and TGF-β [61, 62].

A role for platelets in cell proliferation is suggested by numerous studies linking various platelet constituents, such as PDGF, P-selectin, and PAI-1, with the processes of atherosclerosis and neointimal proliferation [63–66]. Despite these experimental links, clinical studies have failed to demonstrate any significant effect on direct or indirect endpoints of cellular proliferation with GPIIb/IIIa antagonist use [67].

The agents

A complete list of all GP IIb/IIIa antagonists in use and many under clinical development is shown in table 2. In this section, we will concentrate largely on the three parenteral agents that are currently approved for clinical use, with a brief description of oral GP IIb/IIIa antagonists. We will discuss the pharmacology of these agents, and explore the spectrum of their in vitro and in vivo effects. Finally, the impact of these agents on clinical endpoints from randomized clinical trials of patients undergoing percutaneous coronary intervention (PCI) or with acute coronary syndrome (ACS) will be summarized.

Pharmacology: parenteral agents

Parenteral GP IIb/IIIa antagonists in clinical use are broadly divided into two groups: antibodies and small molecules. The latter group may be subdivided into synthetic peptides and synthetic non-peptides. Currently, three parenteral agents, abciximab (antibody), eptifibatide (synthetic peptide), and tirofiban (synthetic non-peptide) are currently approved for clinical use. Table 3 outlines the major pharmacodynamic and pharmacokinetic properties of these agents. These parenteral agents have been used for the short-term treatment of patients undergoing PCI or with ACS. The goal of treatment is to aggressively inhibit $\alpha_{IIb}\beta_3$ receptor function for a short time (24–72 h) during the most vulnerable period of susceptibility to platelet thrombosis.

The initial dosing regimens for each of these three agents were developed using data from in vivo studies using abciximab, which demonstrated that platelet aggregation induced by 20 µmol/l ADP was not inhibited or minimally inhibited at ≤50% receptor blockade, partially inhibited from 50 to 80% receptor blockade, and eliminated at ≥80% receptor blockade [68, 69]. Animal studies further demonstrated that ≥80% receptor blockade by abciximab effectively inhibited thrombus formation in the setting of a severe thrombogenic stimulus [69].

Table 2. List of glycoprotein IIb/IIIa inhibitors currently in phase II/III studies or FDA approved.

Name (trade name)	Manufacturer	Route	Status
Monoclonal antibodies			
Abciximab (ReoPro)	Centrocor	parenteral	FDA approved: PCI, ACS
TM 337	Yamanouchi	parenteral	phase II
Peptide			
Eptifibatide (Integrelin)	COR therapeutics	parenteral	FDA approved: PCI, ACS
Small Molecules			
Tirofiban (Aggrastat)	Merck & Co	parenteral	FDA approved: ACS
Lamifiban	Hoffman-La Roche	parenteral	phase III, ACS
Fradafiban	Boehringer Ingelheim	parenteral	phase II, ACS
Xemlofiban	Searle	oral	phase II, ACS
Orofiban	Searle	oral	phase II, ACS
Sibrafiban (Xubrix)	Hoffman-La Roche	oral	phase II, ACS
Roxifiban	DuPont Merck	oral	phase II, ACS
Lotrafiban	SmithKline Beecham	oral	phase II, ACS, CBVD
Ledrafiban	Boehringer Ingelheim	oral	phase II, ACS
SR-121787	Sanofi	oral	phase II, ACS

Reproduced from Topol et al. [173] with permission. ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; CBVD, cerebrovascular disease.

Table 3. Dosing, pharmacokinetic and pharmacodynamic characteristics of FDA approved GB IIb/IIIa inhibitors.

Antagonist	Abciximab	Eptifibatide	Tirofiban
Receptor Selectivity			
$\alpha_{IIb}\beta_3$	+++	+++	+++
$\alpha_v\beta_3$	+++	0	+
MAC-1/$\alpha_M\beta_2$	++	0	0
K_d (nmol/l)	5	15	120
Plasma half-life	26 min	2.5 h	2 h
Clearance	protein catabolism	renal excretion	renal excretion
Dosing			
PCI			
Bolus	0.25 mg/kg per minute	double bolus 180 µg/kg (10 min apart)	10 µg/kg
Infusion	0.125 µg/kg per minute × 12 h	2 µg/kg per minute × 20–24 h	0.15 mµg/kg per minute × 18–24 h*
ACS			
Bolus	no recommendet[a]	180 µg/kg over 30 min	0.4 µg/kg over 30 min
Infusion		2.0 µg/kg per minute up to 72 h	0.1 µg/kg per minute 48–108 h
Renal dysfunction			
Creatinine ≥ 2 mg/dl	no adjustment required	135 µg/kg bolus over 30 min 0.5 µg/kg per minute infusion	0.2 µg/kg over 30 min 0.05 µg/kg per minute infusion[b]
Creatinine ≥ 4 mg/dl	no adjustment required	contraindicated	

ACS, acute coronary syndrome; PCI: percutaneous intervention.
* Not approved but PCI trials have used this dosing regimen.
[a] Use of abciximab for ACS in absence of planned PCI not recommended.
[b] Dose of tirofiban for all patients with creatinine clearance < 30 ml/min.

Receptor occupancy studies are significantly more complicated for small-molecule antagonists due to the lower affinity and rapid off-rate of these agents, which makes radiometric analyses unreliable. While one can determine the binding of specific antibodies to LIBS (e.g., D3 antibody) as a measure of receptor occupancy, these studies have been rarely performed. Instead, the effects of these agents (as well as abciximab) have been examined using the following approaches.

1) Inhibition of platelet aggregation using the traditional optical turbidimetric method or the rapid assessment of platelet aggregation using a whole-blood aggregometer (e.g., Ultegra Rapid Platelet Function Assay; Accumetrics) [70]. These methods assume that fibrinogen binding to the $\alpha_{IIb}\beta_3$ receptor represents the only relevant interaction for aggregation and may be more applicable to blood flowing at the velocity found in veins. The inhibition of platelet aggregation by inhibitors, as assessed by tradi-

tional turbidimetric methods, is heavily dependent on the dose and type of platelet agonist. For example, thrombin receptor agonist peptide (TRAP) is a more potent platelet agonist than ADP, requiring higher concentrations of inhibitor to achieve the same level of platelet inhibition.

2) Inhibition of shear-induced platelet aggregation (e.g., Platelet Function Analyzer 100/PFA-100, Dade Behring; Cone and Platelet Analyzer/CPA) [71–73]. These studies may be more applicable clinically as they test platelet function under physiologically relevant flow conditions. This may better reflect $\alpha_{IIb}\beta_3$ receptor function on the arterial side of the circulation where $\alpha_{IIb}\beta_3$ receptors play a key role in stabilizing platelet adhesion by binding to vWF.

Antibodies

The first GP IIb/IIIa inhibitor used in humans was a mouse monoclonal antibody that blocked the $\alpha_{IIb}\beta_3$ receptor [74]. In an effort to reduce immunogenicity, the heavy- and light-chain variable regions of this antibody were subsequently attached to the constant regions of human IgG_1 and kappa chains, respectively, to produce a chimeric antibody called abciximab (ReoPro; Centocor). Abciximab is a high-affinity antagonist with a dissociation constant (K_d) of 5 nmol/l, resulting in a predominantly receptor-bound distribution following administration [75]. Abciximab appears to bind to a site on the $\alpha_{IIb}\beta_3$ receptor that is distinct from the ligand-binding site, since it may bind to the receptor even if the binding pocket is occupied by RGD peptides. The inhibitory effect of abciximab may be due to steric hindrance of the ligand access pocket. The cross-reactivity of abciximab with the vitronectin receptor ($\alpha_v\beta_3$), which is found on the surface of endothelial, platelet, and smooth muscle membranes, makes it likely that the β_3 subunit provides the epitope for abciximab binding [76, 77]. Abciximab also interacts with lower affinity to the activated Mac-1 ($\alpha_M\beta_2$) receptor on the surface of myeloid leucocytes (PMNs and monocytes) [76, 77].

Abciximab is currently approved for use in patients undergoing PCI only, although it has been studied in ACS populations [78, 79]. It is administered as a bolus (0.25 mg/kg) followed by a 12-h infusion (0.125 µg/kg per minute). The bolus dose represents ~75% of the total dose administered to an 80-kg individual. Because of the high affinity and rapid on-rate of abciximab for its receptor, two-thirds of the bolus dose becomes bound to the $\alpha_{IIb}\beta_3$ receptor within minutes. The infusion is required because of the slow, yet appreciable, dissociation rate of abciximab from the platelet surface. This dosing regimen has remained remarkably constant through all of the clinical PCI and ACS trials with abciximab.

As with the other GP IIb/IIIa inhibitors, inhibition of fibrinogen binding occurs in a concentration-dependent manner. Within minutes of the bolus dose, the vast majority (~90%) of the $\alpha_{IIb}\beta_3$ receptors are occupied [80]. This degree of receptor occupancy is associated with a median ADP-induced platelet aggregation response of <10% of baseline [81–83]. During the course of the infusion, the extent of inhibition in most patients declines gradually and the consistency of inhibition becomes less uniform, perhaps due to the emergence of the platelet α granule storage pool of receptors [84, 85]. Nevertheless, at the completion of the 12-h infusion, most patients continue to have >80% receptor occupancy, and the median platelet aggregation is ~20% of baseline [81–83, 86]. Abciximab has a slow half-time rate of dissociation from the $\alpha_{IIb}\beta_3$ receptor of up to 4 h. Furthermore, abciximab redistributes from platelet to platelet in vivo, as suggested by the unimodal decrease in abciximab binding by flow cytometry [87]. An estimated 29% and 13% of $\alpha_{IIb}\beta_3$ receptors are occupied by abciximab at 8 and 15 days, respectively, following completion of the infusion. Functionally, these pharmacodynamic properties of abciximab result in significant platelet inhibition as assessed by platelet aggregometry at 12 and 24 h post-infusion which return to normal after 72 h [72, 82]. However, platelet function assessment using shear-induced platelet deposition remains abnormal up to 7 days post-infusion [72]. The lengthy low-level receptor blockade may be responsible for the more subtle pharmacologic effects of abciximab and may explain the inconsistency in clinical outcomes observed with this agent compared with other GP IIb/IIIa inhibitors. Because free unbound abciximab is rapidly cleared from the plasma, platelet transfusion rapidly reverses the platelet inhibition produced by abciximab by providing a source of unbound $\alpha_{IIb}\beta_3$ receptors capable of restoring hemostasis.

Peptides

Eptifibatide (Integrilin; COR therapeutics) is a synthetic disulfide-linked cyclic heptapeptide antagonist whose template was provided by barbourin, a 73-amino acid peptide, and a member of a family of peptides found in snake venom called disintegrins [88]. Disintegrins have an RGD sequence that blocks all integrin receptors that recognize this sequence. Unlike other disintegrins, however, barbourin demonstrates a high specificity for the $\alpha_{IIb}\beta_3$ receptor conferred by its Lys-Gly-Asp (KGD) sequence. Eptifibatide similarly contains this KGD sequence and was modified to provide a significantly improved safety and pharmacologic profile over the natural peptide for clinical use, while maintaining its high specificity for the $\alpha_{IIb}\beta_3$ receptor. Eptifibatide binds to the ligand-binding pocket of the $\alpha_{IIb}\beta_3$ receptor that mediates binding to fibrinogen and vWF. Recently, Basani et al. [89] demonstrated that the tetrapeptide RGDS binds to the third and fourth amino-terminal repeat sequences of the α subunit and induces an allosteric change in a separate portion of the $\alpha_{IIb}\beta_3$ receptor preventing fib-

rinogen binding [89]. Eptifibatide may act in a similar manner.

Eptifibatide is a low-affinity inhibitor compared to abciximab (K_d 120 nmol/l), with a rapid onset of action. The drug dissociates rapidly from the receptor leading to significantly higher circulating pools of unbound eptifibatide as compared with abciximab [75]. In the plasma, 25% of the drug is bound to plasma proteins while the remainder constitutes the pool of pharmacologically active drug. Eptifibatide is primarily cleared by the kidney, making dose adjustment necessary in patients with significant renal dysfunction.

Eptifibatide is currently licensed for use in patients with ACS and in patients undergoing PCI. Unlike abciximab, there have been significant refinements in the dosing of eptifibatide since the initial phase II dose-finding studies. These studies suggested a bolus dose of 135 µg followed by an infusion of 0.5 µg/min provided adequate inhibition of platelet function. However, the sample anticoagulant in these studies was the calcium chelator sodium citrate, which lowers the fibrinogen-binding affinity of the $\alpha_{IIb}\beta_3$ receptor and facilitates eptifibatide binding. The result was to artificially exaggerate the ex vivo effect of eptifibatide compared to the in vivo clinical effect [90]. Subsequent studies increased both the bolus dose (to 180 µg/kg) and infusion rate (to 2 µg/kg per minute) for treatment of patients with ACS [91], and used a double-bolus (180 µg/kg repeated at 10 min) and infusion (2 µg/kg per minute) regimen for patients undergoing PCI [92]. The infusion is generally continued for 48–72 h in patients with ACS and for 24 h in patients undergoing PCI. Most of the clinically relevant in vivo human data on the ability of eptifibatide to inhibit platelet function pertains to the 180 µg/kg bolus and 2 µg/kg per minute infusion regimen. Within 10 min of this bolus and infusion regimen, there is >80% inhibition of platelet function as assessed by the RPFA or standard light transmission turbidimetric aggregometry using 20 µmol/l ADP as the platelet agonist [82, 83, 86]. A recent study of patients with ACS suggests a transient recovery in platelet aggregation between 1 and 4 h post-bolus [93]. Most other studies report maintainence of inhibition of >80% of platelet function throughout the duration of the infusion [82, 83, 86]. Due to the rapid dissociation of the drug, restoration of normal platelet function using standard aggregometry techniques occurs within 4 h of cessation of the infusion.

Pharmacodynamic and pharmacokinetic data with the double-bolus and infusion regimen of eptifibatide are more limited. However, the data from patients undergoing PCI suggests that this regimen eliminates the transient loss of platelet inhibition seen with the single-bolus and infusion regimen [94]. It also produces more adequate $\alpha_{IIb}\beta_3$ receptor blockade (~80%) and inhibition of platelet aggregation (>90%).

Synthetic non-peptides

Tirofiban (Aggrastat, Merck) is a synthetic non-peptide tyrosine derivative that mimics the RGD integrin recognition sequence [95, 96]. Like eptifibatide, this agent competitively inhibits platelet aggregation by occupying the binding pocket on the $\alpha_{IIb}\beta_3$ receptor, and highly selectively inhibits the $\alpha_{IIb}\beta_3$ receptor [75]. It is a lower-affinity antagonist (K_d 15 nmol/l), with the unbound fraction of drug accounting for 35% of the total circulating pool. Tirofiban has a rapid 'off' rate (dissociation half-time of ~10 s). Clearance of the drug is primarily via the renal route, requiring dosing adjustment for patients with renal dysfunction.

Tirofiban is licensed for use in patients with ACS, but has been used in studies of patients undergoing PCI [97, 98]. The usual dosing regimen for patients with ACS is a bolus dose of 0.4 µg/kg per minute given over 30 min followed by an infusion of 0.1 µg/kg. In patients undergoing PCI, the bolus dose that has been used is 10 µg/kg given over 30 min (i.e., 0.33 µg/kg per minute), with an infusion rate of 0.15 µg/kg. These regimens were defined in dose-finding studies that examined the ability of tirofiban to inhibit platelet aggregation using 5 µmol/l of ADP, in contrast to 20 µmol/l ADP used in studies with abciximab and eptifibatide [99]. This has led to some reservations about the adequacy of platelet inhibition with the currently accepted dosing regimens of tirofiban. Despite these reservations, most studies assessing platelet inhibition by tirofiban, using the regimens outlined above, demonstrate >90% inhibition of platelet aggregation using 20 µmol ADP within 10 min of the bolus dose, with this level of inhibition maintained throughout the course of the infusion [82, 86]. Like eptifibatide, normal hemostatic function is restored within 3–4 h following discontinuation of the infusion.

Pharmacology: oral IIb/IIIa inhibitors

Of the many oral IIb/IIIa inhibitors that have been investigated, four have been evaluated in phase III clinical trials: sibrafiban, lotrafiban, orbofiban, and xemilofiban [100–103]. These are non-peptide pro-drugs that undergo one or more enzymatic steps during hepatic conversion to the active form. Oral bioavailability of these agents is generally low (10–40%) [104]. Their half-lives vary from 4 h in the case of xemilofiban to 11 h for sibrafiban. This resulted in twice daily dosing for all of these agents except xemilofiban, which was administered three times daily. Pharmacokinetic studies demonstrated a correlation between plasma levels of the active metabolite of these agents and the degree of platelet inhibition [105, 106]. High- and low-dose arms were used in the clinical trials with the aim of achieving between 30–60% and 50–80% inhibition of platelet aggregation, respectively, as assessed using 20 µmol ADP. However, because

of the characteristic peak and trough in plasma concentrations after each dose [105, 106], the actual measured inhibition of platelet aggregation was found to fall considerably short of these goals immediately prior to the next scheduled dose [107].

In vitro and in vivo effects of glycoprotein IIb/IIIa antagonists

The dominant effect of GP IIb/IIIa antagonists is the inhibition of platelet aggregation. This effect likely explains most of the beneficial clinical effects seen in patients treated with these agents. As suggested in the section on $\alpha_{IIb}\beta_3$ receptor function, however, these agents may have other important platelet and non-platelet effects. Some of these effects may be beneficial while others may be harmful. Indeed, recent clinical trials have demonstrated that these agents do not always confer clinical benefit despite the rational basis for therapeutic benefit based on pathophysiologic mechanisms. The current section summarizes the available evidence from in vitro and in vivo studies of the effect of GP IIb/IIIa antagonists on events other that the simple inhibition of platelet aggregation. In addition to those listed, there remain a host of RGD motifs and RGD binding sites in multiple proteins that are important for cardiovascular and non-cardiovascular function that may be influenced by these agents and exert heretofore unrecognized effects [108].

Thrombus size and architecture

The effect of inhibition of platelet aggregation is to reduce thrombus size. While in vitro evidence of this effect exists [109], more powerful in vivo evidence supporting this effect of GP IIb/IIIa antagonists has been reported. Abciximab and tirofiban have both been shown to reduce thrombus burden and/or increase the incidence of complete thrombus resolution as assessed by coronary angiography in the setting of unstable angina, non ST-elevation myocardial infarction (MI), and ST-elevation MI [110–112]. Using blood samples from 23 patients with acute MI treated with abciximab, Collet et al. [113] recently confirmed the ability of abciximab to alter the architecture of platelet rich clots (PRCs). Abciximab reduced both the surface area and the rigidity index (G′ dynes/cm^2) of PRCs in an ex vivo model. Both of these effects are likely to promote fibrin exposure within the clot, with enhancement of both endogenous and therapeutic fibrinolysis [114].

Anti-inflammatory effect

An anti-inflammatory effect for GP IIb/IIIa antagonists has been suggested based on basic science studies demonstrating the numerous potential pro-inflammatory actions of activated platelets. Recent evidence suggests that this effect may be real. Neumann et al. [115] initially examined the effect of abciximab on the interaction between platelets and myeloid leukocytes (neutrophils and monocytes) and the surface expression of αMβ_2/Mac-1 in patients undergoing PCI following a recent MI. Abciximab did not affect the percentage of myeloid leukocytes with attached platelets but did reduce the amount of platelets incorporated into platelet-leukocyte aggregates, as determined by the intensity of GP Ibα immunofluoresence [115]. This was associated with a decrease in Mac-1 expression on monocytes and neutrophils within 24 h of reperfusion with abciximab that persisted up to 72 h following intervention. This effect of abciximab is likely not only to limit the bulk and adhesiveness of platelet-leukocyte aggregates and thus reduce microvascular obstruction but also to inhibit inflammation. Neumann et al. [86] demonstrated a similar effect of tirofiban and eptifibatide on platelet-leukocyte interactions and Mac-1 expression by myeloid leukocytes as compared with abciximab. However, Furman et al. [116] reported that both tirofiban and eptifibatide, but not abciximab, in the absence of platelet agonist promoted platelet-leukocyte aggregation, an effect that was potentiated in the presence of agonist.
More direct evidence of an anti-inflammatory effect of these agents comes from a recent study by Lincoff et al. [117]. In a 160-patient subset of the EPIC trial, where patients underwent high-risk angioplasty, abciximab had an attenuating effect on the peri-procedural elevation of systemic inflammatory markers. Levels of C-reactive protein, IL-6, and TNF-α within the first 24–48 h following angioplasty were reduced by 32, 76, and 100%, respectively, in patients treated with abciximab compared to placebo. These reductions were independent of an effect of abciximab on reducing ischemic events. The effect of tirofiban or eptifibatide on these markers has not been reported.

Platelet secretion

The products of platelet secretion mediate important effects on platelet activation, inflammation, atherogenesis, thrombin generation, and fibrinolysis (see above). Given the beneficial effects of GP IIb/IIIa antagonists on platelet aggregation, and the central role of the $\alpha_{IIb}\beta_3$ receptor in platelet activation, these agents were initially assumed to also have a beneficial effect on other components of platelet activation such as platelet secretion. However, closer examination of the effects of these agents on platelet secretion has yielded somewhat inconsistent results.
Regarding parenteral agents, several in vitro studies have demonstrated either a mild or absent antisecretory effect of abciximab [118–120], eptifibatide [120], and

tirofiban [120, 121] at concentrations shown to maximally inhibit platelet aggregation. Others have suggested a paradoxical potentiation of platelet secretion with abciximab and eptifibatide [121] that was not seen with non-peptide inhibitors. Neumann et al. [86] examined the percentage of p-selectin-positive platelets in human patients undergoing PCI and found no significant difference with abciximab, eptifibatide, or tirofiban. The precise effect of oral GP IIb/IIIa antagonists on platelet secretion is unclear. It is difficult to discern whether any potential pro-secretory effect of these agents represents a true effect, or alternatively a partial agonist effect, which has been postulated to occur at dose throughs of oral agents.
In summary, these studies raise the suggestion of a discoupling between the anti-aggregatory and anti-secretory effects of parenteral agents. It remains uncertain whether in-vitro studies truly reflect the in-vivo action of these agents on platelet secretion.

Apoptosis

Recent studies have implicated RGD peptides and RGD-mimetic $\alpha_{IIb}\beta_3$ receptor blockers in promoting cell apoptosis through effects on enzymes of the caspase family, specifically caspase-3, and perhaps caspase-1 [108, 122]. Initially, this effect was recognized in resting, activated, and transformed leukocytes [122]. More recently, Adderly and Fitzgerald [108] reported the induction of apoptosis within 24–72 h of exposure of rat cardiomyocytes to orbofiban and xemlofiban, but not abciximab or eptifibatide.
Caspases are derived from their precursor and catalytically inactive procaspases, and are commonly the ultimate signal in response to factors that induce apoptosis [123]. They activate a number of targets including nuclear enzymes involved in DNA degradation. Evidence suggests that apoptosis induced by RGD peptides or mimetics results from passive diffusion of these agents into the cell cytoplasm, as opposed to ligand binding to the integrin receptor. Binding of the RGD peptide or RGD mimetic to an RGD-binding motif near the processing site in pro-caspase 3 is postulated to induce a conformational change in the pro-enzyme, which leads to oligomerization and autoprocessing of the enzyme. The inability of abciximab to cross the plasma membrane of the cardiomyocyte, and the presence of a KGD as opposed to an RGD sequence in eptifibatide, may explain the absence of an effect by these agents on caspase 3 activity. It should be noted that the plasma concentrations of orbofiban and xemlofiban that induced apoptosis in the in vitro experiments by Adderly and Fitzgerald [108] were far in excess of those required to inhibit platelet aggregation. However, hypoxia appears to potentiate the pro-apoptotic activity of orbofiban, raising the possibility that ischemic tissues may be susceptible to the pro-apoptotic effects of this agent at the concentrations found in vivo.

Anti-coagulant effect

A number of studies support an anti-coagulant effect for GP IIb/IIIa antagonists. This is not surprising, given the link between platelets and components of the intrinsic and extrinsic arms of the coagulation cascade. Moliterno et al. [124] observed a significant prolongation of the ACT (an automated assessment of a modification of the clotting time used to monitor heparin dosing in the interventional laboratory) in patients receiving abciximab compared to placebo during PCI. Several in vitro studies have provided insights into the potential mechanism for this anti-coagulant effect. In vitro models demonstrate that GP IIb/IIIa antagonsists reduce thrombin generation, as reflected by reduced levels of thrombin, thrombin-antithrombin III complex, and fibrinopeptide$_{1+2,}$ in response to platelet agonist [125]. Additional effects that have been demonstrated include the inhibition of pro-thrombinase complex assembly, pro-coagulant microparticle formation, and exposure of phospholipid on the platelet membrane [55, 125–128]. Furman et al. [120] reported a greater anti-coagulant effect with abciximab compared with either eptifibatide or tirofiban. While one study suggested that the anti-coagulant effect of abciximab was due to blockade of both the $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$ receptor [125], subsequent studies suggest that the effect is due exclusively to blockade of the $\alpha_{IIb}\beta_3$ receptor alone [120, 129]. This leaves the perplexing question as to how antibody and small molecule inhibitors mediate this differential anti-coagulant effect. Whether this phenomenon is clinically relevant is also unclear.

Partial agonist effects

The ability of GP IIb/IIIa antagonists to act as both competitive antagonists and partial agonists of integrin function was recognized over a decade ago, long before the potential pro-thrombotic effects of these agents were reported in clinical studies [130]. Both antibody and small-molecule GP IIb/IIIa antagonists have been demonstrated in in vitro studies to bind the $\alpha_{IIb}\beta_3$ receptor in both the activated and quiescent states, in contrast to endogenous ligand that binds only to the activated receptor. Both groups of agents have been demonstrated to have an intrinsic activating property [131], although a recent report contradicts this finding [132]. If the former hypothesis is true, GP IIb/IIIa antagonists may bind the $\alpha_{IIb}\beta_3$ receptor in the resting state and promote the formation of a high-affinity state, through induction of a conformational change in the receptor. Dissociation of the inhibitor may thus enable the receptor to bind soluble fibrinogen in the absence of agonist-induced platelet activation. Therefore,

at low concentrations, GP IIb/IIIa antagonists may induce platelet aggregation, while at high concentrations they inhibit platelet aggregation.

During therapy with parenteral agents for PCI and ACS, where dosing of the GP IIb/IIIa antagonist achieves potent platelet inhibition that is consistently maintained for a relatively short period of time (24–72 h), any partial agonist activity is unlikely to have a clinical effect. In contrast, during therapy with oral GP IIb/IIIa antagonists, where treatment duration is considerably longer (weeks/months) and the dosing schedule leads to peaks and troughs in the plasma drug concentration, partial agonist activity during trough periods may indeed be clinically relevant. The results from basic studies of small subgroups of patients enrolled in the oral GP IIb/IIIa inhibitor trials have been inconsistent, however. In 15 patients enrolled in the OPUS study, Holmes et al. [133] demonstrated that after 14 days of therapy with orbofiban, platelet fibrinogen binding in response to 0.2 μM ADP was increased significantly compared with assessment at baseline. Sibrafiban, which was subject to the same fluctuations in plasma drug concentrations, and had a similar adverse clinical profile compared to orbofiban, did not appear to demonstrate this partial agonist effect as assessed by platelet p-selectin expression [134]. The partial agonist property of currently available oral GP IIb/IIIa antagonists is likely responsible for some of the untoward clinical events noted in some clinical trials to be discussed below.

LIBS formation

Like fibrinogen and other $\alpha_{IIb}\beta_3$ receptor ligands, GP IIb/IIIa antagonists appear to induce the formation of LIBSs by inducing conformational change in the $\alpha_{IIb}\beta_3$ receptor. In addition, they have also been shown to induce the disappearance of epitopes on the $\alpha_{IIb}\beta_3$ receptor (ligand-attenuated binding sites) [107]. Gawaz et al. [135] demonstrated in blood drawn from patients undergoing PCI who received abciximab that there was a dose-dependent increase in the exposure of LIBSs LIBS-1and PMI-1, which persisted for at least 96 h following completion of the infusion [135]. The expression of LIBSs with abciximab was approximately one-third that produced by the G**RGD**SP peptide ligand. The degree of LIBS expression by abciximab was inversely related with the peripheral platelet count, which suggests a possible role for this neoepitope in abciximab-induced thrombocytopenia. Jennings et al. [136] further demonstrated the differential expression of a further LIBS (D3) on the GP IIIa subunit (residues 422–490) following incubation of platelets with abciximab which produced no measurable increase in D3 expression, and tirofiban or eptifibatide which produced a significant increase in D3 expression. The latter observation raises the interesting possibility that different GP IIb/IIIa inhibitors may exert differential effects on platelet function by inducing specific conformational changes in the $\alpha_{IIb}\beta_3$ receptor [137].

Clinical efficacy

Since the first large GP IIb/IIIa inhibitor trial in 1994, these agents have been studied in ~14 large clinical trials (>1000 patients) involving over 100,000 patients (fig. 4, 5). These trials have focussed on three broad areas of application in cardiovascular medicine: PCI, ACS (excluding ST-elevation MI), and ST-elevation MI. These clinical studies have largely measured ischemic endpoints such as MI, and need for revascularization (urgent and/or elective). Because of their size, they have also been able to report clinically meaningful mortality data. Many smaller studies and post hoc analyses of larger studies have been performed which provide important insights into the mechanism of action and the optimal clinical utility of these agents.

Coronary intervention

The greatest therapeutic impact of GP IIb/IIIa antagonists has been demonstrated in patients undergoing PCI. All of the large clinical trials in the PCI population have used these agents as prophylactic therapy to prevent ischemic complications and have demonstrated a remarkably consistent beneficial effect in reducing ischemic endpoints. In a pooled analysis of all placebo-controlled PCI trials, the absolute and relative risk reduction in the composite 30-day endpoint of death/MI with GP IIb/IIIa use was 2.9 and 34%, respectively. These represent the largest absolute and relative risk reductions seen in any of the GP IIb/IIIa trials. The benefits have been consistent across a broad range of patient populations in terms of clinical presentation, lesion morphology [138, 139], and interventional technique (i.e., angioplasty, stenting, atherectomy) [140].

The major clinical endpoints impacted in PCI trials are that of MI, predominantly Q wave and large non-Q wave, and urgent revascularization. Temporally, these endpoints are reduced predominantly in the first 48 h following intervention, with a further modest benefit over the 48-h to 30-day period. The importance of the endpoint of MI has been borne out by studies demonstrating a significantly increased mortality at 1 year in patients with CK-MB elevations both peri-procedurally and during early follow-up (≤30 days) [141–143]. Placebo-controlled trials have suggested a more robust and sustained reduction in MI and urgent revascularization with abciximab, as opposed to eptifibatide [92, 144] and tirofiban [97], in patients undergoing either angioplasty [145, 146] or stenting [147]. The 30-day outcome of the recent TARGET trial [98],

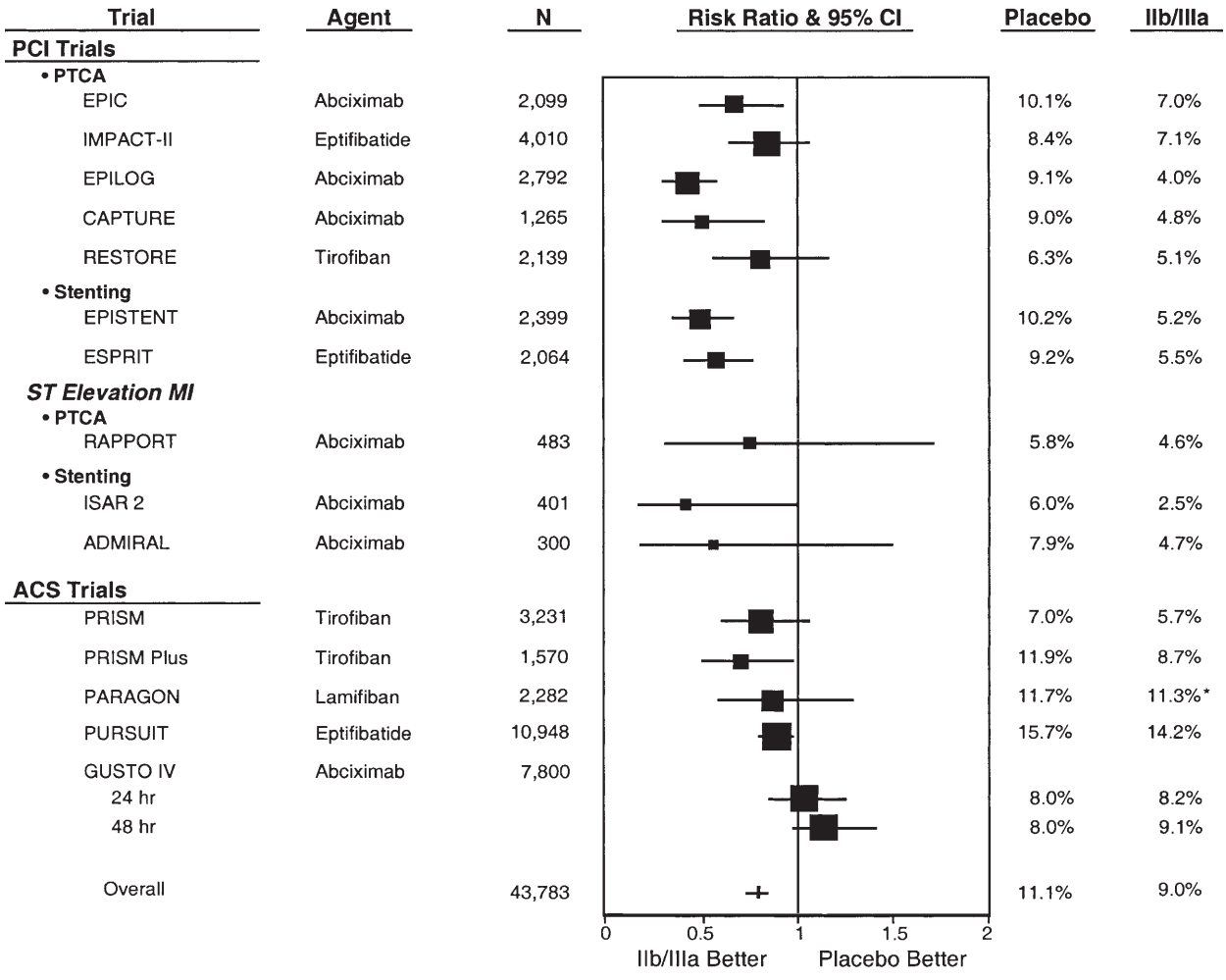

Figure 4. Death or non-fatal MI outcomes at 30 days in randomized placebo-controlled trials of parenteral GP IIb/IIIa blockers. Risk ratio with 95% CI, size of RR box being proportional to total sample size. Frequency of death or non-fatal MI in columns 5 and 6.

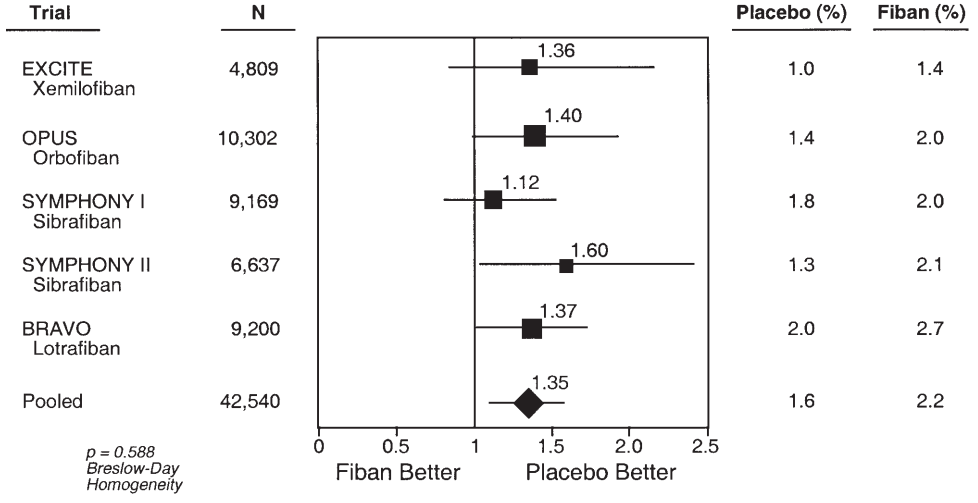

Figure 5. Death at 30 days in trials of oral GP IIb/IIIa trials. Risk ratio with 95% CI, size of RR box being proportional to total sample size.

which is the first head-to-head study of GP IIb/IIIa use during PCI, confirmed the superiority of abciximab over tirofiban in reducing these endpoints. Reduction in mortality has been seen only with abciximab, and becomes manifest only during longer-term follow-up. The EPISTENT study was the first to demonstrate a statistically significant reduction in mortality with abciximab use in PCI. This benefit only became apparent during the analysis of the data at 1-year follow-up (1% versus 2.4%, $p = 0.037$) [148]. The EPIC and EPILOG studies currently have follow-up data for 7 years and 4.5 years post-PCI, respectively, and each demonstrates a trend toward a reduction in mortality with abcixiamb (EPIC 17.4% versus 20.4%, EPILOG 8% versus 9.5%). Despite reports from the earliest EPIC study that abciximab reduced the overall rate of target vessel revascularization after balloon angioplasty at 6-month follow-up [149], subsequent studies have failed to reproduce these findings with either abciximab or small-molecule agents. One important exception is the diabetic population in whom adjunctive abciximab use with stenting was associated with a halving of the 6-month revascularization rate (16.6% stent alone versus 8.1% for stent plus abciximab) [150]. This raises the interesting hypothesis that abciximab may influence the process of restenosis in this sub-population of patients. Somewhat surprisingly, the analysis of the diabetic population in the ESPRIT study, which used adjunctive eptifibatide with stenting in a somewhat lower risk population than that in the EPISTENT population, found the opposite effect [target vessel revascularization rate (TVR) of 10.2% in placebo group versus 14.9% in eptifibatide group; unpublished data]). The 6-month follow-up of the TARGET trial showed similar rates of TVR in diabetics, suggesting the primacy of $\alpha_{IIb}\beta_3$ receptor inhibition in driving clinical outcomes.

Acute coronary syndromes – unstable angina and non-ST elevation MI

Considerable heterogeneity exists among the large clinical trials testing empiric GP IIb/IIIa antagonist use in patients with ACS. There was marked variation in patient entry criteria, type of agent used, duration of therapy, use of adjunctive agents, approach to diagnostic angiography and revascularization procedures, and the timing and composition of the primary endpoint. The results of the recently reported GUSTO IV study [78] have added further complexity to the application of the use of GP IIb/IIIa antagonists in patients with ACS.

The small-molecule inhibitors, tirofiban and eptifibatide, have demonstrated efficacy when used as a component of medical therapy alone or when combined with PCI or surgical revascularization [91, 151, 152]. A meta-analysis of all ACS trials (i.e., including all treatment strategies) using small-molecule inhibitors reported no significant difference in mortality with GP IIb/IIIa antagonist use, but did show a significant reduction in the composite endpoints of death/MI, and death/MI/revascularization at 48–96 h, 30-day, and 6-month follow-up [153]. The temporal pattern of benefit is similar to that observed in PCI trials, with most benefit seen in the first 48 h. However, the benefit is considerably more modest than that observed in PCI trials, with an overall absolute and relative risk reduction in the incidence of death and MI at 30 days of 1.4% and 11% respectively [154]. Post hoc analyses have shown that the benefits are largely seen in patients who undergo either PCI or surgical revascularization [155, 156], and in those who demonstrate evidence of myonecrosis (reflected by elevated troponin levels) [154, 157].

The role of abciximab in patients with ACS is less certain. Abciximab has shown benefit in patients with ACS who undergo PCI following a prolonged infusion (i.e., 24 h) of abciximab. The benefit of abciximab reflected the composite reduction in death and MI observed both during the medical treatment phase and following PCI [79]. However, the recently reported GUSTO IV study demonstrated no benefit of treatment with abciximab when used as medical therapy alone for patients with ACS [78]. There was even a trend toward an adverse effect in the bolus and 48-h abciximab infusion arm of the study with an increased incidence of the endpoints of death and MI. At 1-year follow-up, the death rate for the 48-h abciximab treatment group was persistently higher. Many potential explanations for the observed difference in outcome between this and previous studies have been suggested. The most reasonable explanation for the deleterious effect of prolonged abciximab administration is the partial agonist action caused by sub-optimal $\alpha_{IIb}\beta_3$ receptor blockade. Patients with an elevated C-reactive protein at baseline had a significantly increased rate of death at 1-year follow-up, again reinforcing the likelihood of a paradoxical platelet activation mechanism.

Acute MI

Despite the success of the large fibrinolytic trials of the 1980s and early 1990s in the treatment of ST-elevation MI, a number of limitations of fibrinolytic therapy became clear. These agents achieved TIMI 3 flow in only ~50% of patients, and early reinfarction occurred in 4–6% of cases [158, 159]. The failure to achieve higher rates of reperfusion has been termed 'thrombolytic resistance.' The basis for this resistance is thought to be explained by the ability of these agents to act only on the fibrin component of thrombi, as well as their direct and indirect (by exposing clot-bound thrombin) platelet-activating effect [160]. The demonstrated link between mortality benefit and improved rates of restoration of normal antegrade flow (TIMI 3) [161, 162] prompted the search for newer therapies to achieve this surrogate endpoint

with the hopes of improving survival. The benefits of aspirin (a relatively weak anti-platelet agent) on mortality and ischemic endpoints in the ISIS-2 trial [163] suggested that more potent anti-platelet agents such as GP IIb/IIIa antagonists in combination with fibrinolytic agents might achieve improved rates of reperfusion with less reocclusion, resulting in improved survival.

This hypothesis has been tested in a number of pilot studies. Initially, full-dose thrombolytic therapy in combination with GP IIb/IIIa antagonism was used. While achieving improved patency rates, bleeding complications were increased [164–166]. Subsequent studies examined the role of half-dose thrombolytic/GP IIb/IIIa antagonist combinations [167, 168]. These have continued to report improved epicardial patency rates together with evidence of improved myocardial perfusion [169], but with acceptable bleeding rates.

The GUSTO V study is the first large trial examining clinical endpoints using the combined thrombolytic/GP IIb/III antagonist approach [170]. No mortality benefit was seen with abciximab/half-dose reteplase versus full-dose reteplase treatement. However, there was a significant reduction in reinfarction, recurrent ischemia and urgent revascularization using combination therapy. In addition, there was a consistent trend toward reduction in a number of other secondary complications of myocardial infarction. The lack of a mortality benefit with this combination approach to therapy is disappointing given the results of the pilot studies which demonstrated improved rates of TIMI 3 flow using this approach and previous angiographic sub-studies linking this surrogate endpoint with improved survival. The ASSENT III trial, which tested the combination of tenecteplase (TNK) and abciximab corroborated the results of GUSTO V with a marked reduction of reinfarction, no reduction of mortality, and an excess of bleeding complications [171].

Oral GP IIb/IIIa inhibitors

The use of oral GP IIb/IIIa inhibitors to achieve more prolonged and potent platelet inhibition following PCI or ACS is supported by a number of observations. Ischemic complications following PCI and ACS continue to occur with a high frequency beyond the period of platelet inhibition produced by intravenous agents. The less impressive results of PCI trials using parenteral small-molecule competitive inhibitors (eptifibatide and tirofiban) was hypothesized to result from the rapid loss of anti-platelet effect following discontinuation of the drug infusion. Platelets from patients with ACS have also been demonstrated to have a high rate of spontaneous activation as well as an exaggerated response to exogenous agonist for up to 4 weeks following initial presentation [134].

Despite the aforementioned observations, studies to date using oral GP IIb/IIIa inhibitors in the setting of PCI and ACS have consistently failed to demonstrate benefit [100–103]. A recent meta-analysis of four of these studies demonstrated a significantly increased risk in mortality and major bleeding, and a neutral effect on MI associated with the use of these agents [172]. The overall increase in mortality for all five oral IIb/IIIa inhibitor studies is 35% ($p=0.002$) (fig. 5). In contrast, the need for urgent revascularization was significantly reduced in each of these studies.

Given the tremendous promise of these agents and their potential impact on treatment of cardiovascular disease, the results of these studies have been extremely disappointing. Taken together with the outcomes in GUSTO IV, $\alpha_{IIb}\beta_3$ receptor inhibition of $<80\%$, which occurs with all oral agents and during the extended infusion of abciximab, seems to unleash platelet activation. Furthermore, Phillips and colleagues have demonstrated shedding of platelet CD40 ligand when the $\alpha_{IIb}\beta_3$ receptor is not sufficiently inhibited [D. R. Phillips, personal communication]. This would be expected to markedly exacerbate underlying arterial inflammation and could explain the excess in mortality. The potential pro-apoptotic effect of these agents was previously discussed, but remains strictly a hypothetical explanation at this time [108, 122].

Conclusions

The development of parenteral GP IIb/IIIa antagonists for use in clinical practice represents a major step in our treatment of cardiovascular disease. The parenteral agents improve the safety of PCI in all clinical settings, and when used appropriately have also been shown to improve outcomes in patients with ACS and ST-elevation MI. In contrast, oral GPIIb/IIIa antagonism with the agents currently available has proven to demonstrate toxicity manifest as increased mortality when used in similar patient populations. In aggregate, the narrow therapeutic window for $\alpha_{IIb}\beta_3$ receptor inhibition appears to have been amply demonstrated.

Despite all the advances that have been made in this field, many more challenges need to be addressed. Many questions remain concerning the optimal clinical utility of the agents currently available. Uncertainty still remains about the optimal level of platelet inhibition with these agents, the best method of monitoring therapy, and whether monitoring of therapy can alter clinical outcomes. We need to understand the molecular mechanism of action of these agents with greater detail. The effects of these agents on platelet processes other than platelet aggregation, and non-platelet events that have potentially important clinical implications needs to be explored. This exploration may help explain some of the heterogeneity in clinical efficacy seen with antibody versus small-molecule parenteral GP IIb/IIIa antagonists. It may also an-

swer the critical question of the clinical utility of $\alpha M\beta_2$ and $\alpha_v\beta_3$ receptor inhibition. Answers to these questions will hopefully direct the development of second-generation GP IIb/IIIa antagonists with improved clinical efficacy. Clinical studies using oral GPIIb/IIIa antagonists clearly also raise very serious questions. Further investigation will include examination of the molecular events associated with long-term $\alpha_{IIb}\beta_3$ receptor inhibition, and the development of agents with distinctly different pharmacodynamic and pharmacokinetic properties from those previously tested.

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