Inhibitors of Platelets Glycoprotein IIb/IIIa (GP IIb/IIIa) Receptor: Rationale for their Use in Clinical Cardiology

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Abstract: The glycoprotein IIb/IIIa (GP IIb/IIIa) receptor is the most important receptor involved in platelet aggregation. A stable GP IIb/IIIa inhibition is required when a massive platelet activation triggers thrombosis. Three GP IIb/IIIa inhibitors are currently approved for clinical use: abciximab, tirofiban and integrilin. Their different pharmacodynamic and pharmacokinetic properties reflect a different efficacy in platelet inhibition.

Keywords: Platelet aggregation inhibitors, glycoprotein IIb/IIIa receptor, acute coronary syndromes, atherosclerotic plaque, percutaneous coronary intervention.

THE GLYCOPROTEIN IIB/IIIA RECEPTOR

The glycoprotein IIb/IIIa (GP IIb/IIIa) receptor belongs to the integrin family of heterodimeric adhesion molecules [1]. Integrins are expressed on virtually all cell lineages mediating several physiological responses. Among the integrin subunits family, the combination between $alpha_{IIb}$ and $beta_3$ subunits (GP IIb/IIIa) has been found mainly in cells of the megakaryocyte lineage [2] .

The alfa (130-kDa) subunit consists of a light (25 kDa) and a heavy (105 kDa) chain. The light chain has a short cytoplasmatic tail, a transmembrane region, and a short extracellular domain, whereas the heavy chain is entirely extracellular [3]. The beta (95-kDa) subunit consists of a single polypeptide of 762 amino acids, with a short cytoplasmatic tail, a single transmembrane region, and a large extracellular domain with an intricate disulphide arrangement [4,5]. Divalent cations, mainly calcium, are required to maintain the heterodimeric structure, since subunits are not covalently bound to each other [2,6,7].

The two cytoplasmatic tails entwine each other to form a second binding domain for cytoskeletal molecules or intracellular signaling [1]. The amino terminal of each subunit consists of an 8 x 12 nm globular head, whereas the carboxyl terminal is represented by a 18 nm flexible tail, as shown by electron microscopy [8].

The GP IIb/IIIa receptor (Fig. (**1**)) is the most abundant receptor on the platelet surface, with approximately 50,000 to 80,000 copies. It plays a key role in platelet aggregation, with only minimal involvement in platelet adhesion [9]. In the rare congenital disease of "Glanzmann's thrombasthenia", the absence of GP IIb/IIIa receptors is associated with platelets' inability to aggregate *ex-vivo*, but very mild and infrequent clinical bleeding [10,11]. This receptor primarily binds fibrinogen and prothrombin. Fibrinogen binding occurs on damaged vessel walls and within platelet aggregates, and is involved in development of alfa-granules (internal pool of GP IIb/IIIa) during megakaryocytopoiesis. Binding to prothrombin induces an increase in its rate of conversion to thrombin [12,13].

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Atherosclerotic plaque fissuring with local exposure of sub-endothelial matrix, as occurring in acute coronary syndromes or percutaneous coronary intervention, triggers massive platelet activation with subsequent formation of platelet-rich arterial thrombi. In this setting, effective and complete platelet inhibition is needed to achieve consistent clinical benefits [14,15]. In fact, while under normal conditions platelets circulate without interacting with the endothelium or with other platelets, after activation there is recruitment of alfa granule-GP IIb/IIIa to the external membrane, increasing GP IIb/IIIa receptor concentration on the platelet surface as much as 50% [16]. This internal receptor pool is very important because it is able to support platelet aggregation even if the usual surface-pool of GP IIb/IIIa receptor has been inactivated. [9,14,15,17]. Furthermore, platelet activation leads to a conformational change in the GP IIb/IIIa receptor, raising its affinity to fibrinogen. These conformational changes in general induce protein-receptor binding, and more specifically binding of fibrinogen. After the globular head of GP IIb/IIIa has interacted with fibrinogen, the tails are driven laterally, at a 90° angle to the long axis of fibrinogen [8,18]. With its other end, a fibrinogen molecule is able to bind another GP IIb/IIIa receptor from a different platelet, thus forming a bridge between two platelets [8]. This phenomenon, sustained by phosphorylation of the internal binding domain of the GP IIb/IIIa receptor (so called "inside-outside" signal) [19], is a consequence of the activation of protein kinase C due to potent platelet activators such as thromboxane A2, collagen, norepinephrine, ADP and thrombin [20]. Therefore, as a consequence of activation, there is a sudden burst in both the number and affinity of the GP IIb/IIIa receptors on the platelet surface [21,22]. Subsequently, concomitant increase in intracytoplasmatic calcium ions, change in pH values and contraction of the cytoskeleton lead to progressive recruitment of platelets, clot retraction and thrombus formation [19,23].

The recognition specificity of fibrinogen to the glycoprotein IIb/IIIa receptor is defined by two peptide sequences. The main sequence involved in the binding is the KQAGDV (Lys-Gln-Ala-Gl-Asp-Val) sequence, located at the carboxyl terminal of the gamma chain of fibrinogen [24,25]. This sequence is detectable only in fibrinogen [24-29]. The second sequence is the Arg-Gly-Asp (RGD)

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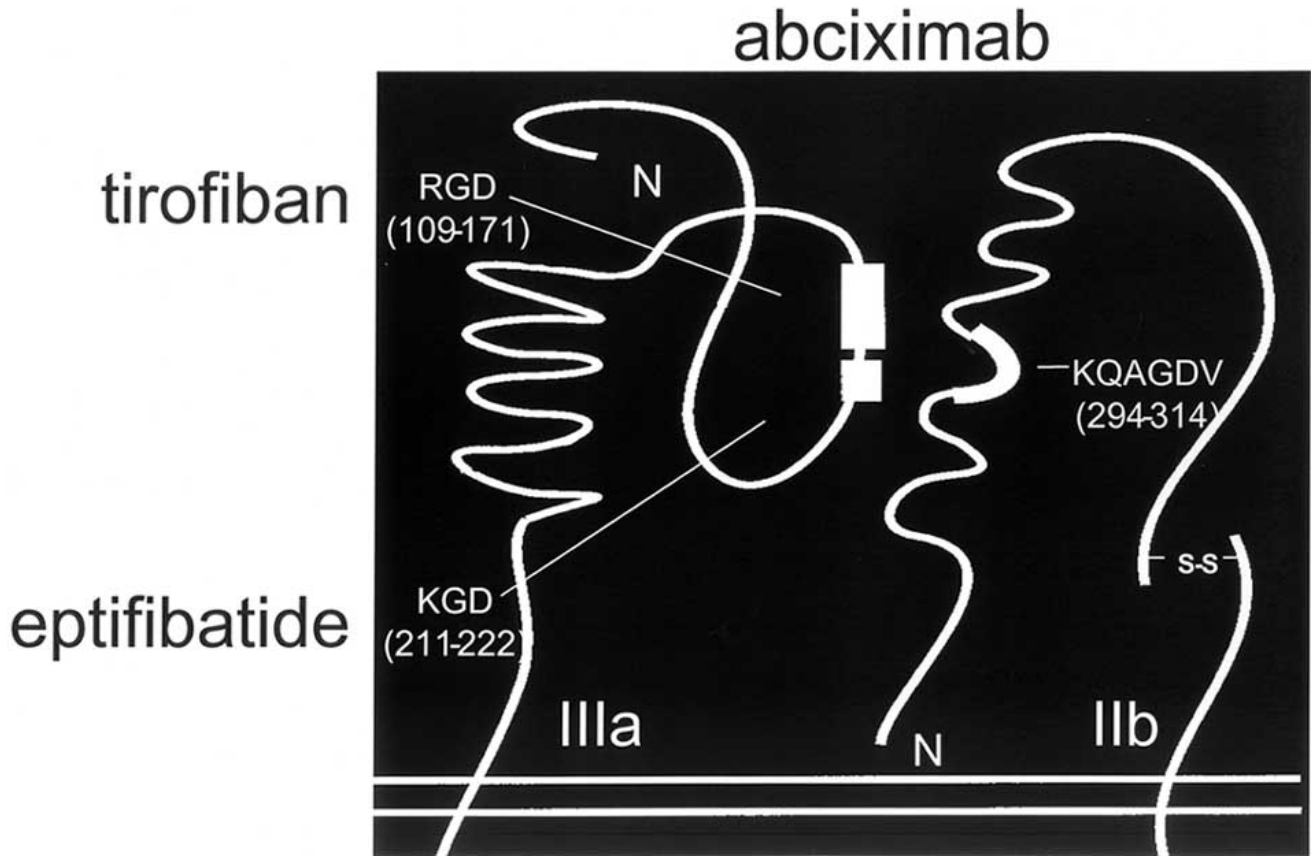

Fig. (1). Schematic depiction of the $\alpha_{IIb}\beta_3$ (GP IIb/IIIa) integrin and distinct ligand-binding sites of principal receptor inhibitors.

sequence [27], which has been initially identified as the adhesive sequence of fibronectin [30], but is also present in von Willebrand factor, vitronectin and fibrinogen which contain two RGD sequences per half molecule [31]. The role of RGD in binding GP IIb/IIIa is still not clear because recombinant fibrinogen lacking these sequences is equally able to bind the receptor [32]. However, introduction of RGD sequences into proteins confers anti-platelet properties [28]. Linear peptides based on the RGD template are less resistant to enzymatic breakdown than cyclic RGD peptides (i.e. MK-852; Fig. (**2a**)), which have a higher potency.

GP IIB/IIIA RECEPTOR INHIBITION

Prompt, stable and complete platelet inhibition is highly desirable in acute coronary syndromes and during percutaneous coronary interventions, both characterized by high thrombotic risk and a pro-thrombotic state.

Ever since clinical benefit was observed with aspirin in these settings [33,34], newer strategies have been evaluated in order to achieve more powerful platelet inhibition. Both preclinical studies and pharmacodynamic evaluation in patients have set the range of >80% inhibition of platelet aggregation as the target for clinically effective antiplatelet activity [35]. The appropriateness of this target has been validated by clinical trials demonstrating both efficacy and safety within this level of platelet inhibition**,** which is associated with a profound decrease in platelet function, regardless of the initial pro-thrombotic stimulus [36]. Furthermore, drug selectivity for the receptor provides an efficient blockade of GP IIb/IIIa without modifications of platelet adhesive properties, minimally affecting normal hemostasis.

Several GP IIb/IIIa antagonists have been developed for this purpose and they can be divided in three main classes: monoclonal antibodies, peptides and small molecules (Fig. (**3**)). Only three of them are currently approved for clinical use by the U.S. Food and Drug Administration: abciximab (monoclonal antibody), eptifibatide (peptide, Fig. (**2b**)) and tirofiban (small molecule, Fig. (**2c**)). All of them are available for intravenous administration. Oral agents (Xemilofiban, Fig. (**2d**), Orbofiban, Sibrafiban) have also been tested with the intent of prolonging the benefits, observed acutely with parenteral compounds, to long-term secondary prevention of ischemic events. Surprisingly, their use was associated with a paradoxical increase in adverse events, such as bleeding and mortality. These effects could be related to the lower level of platelet inhibition achieved with these drugs [37-39]. Therefore, these compounds are not commercially available and will not be discussed further.

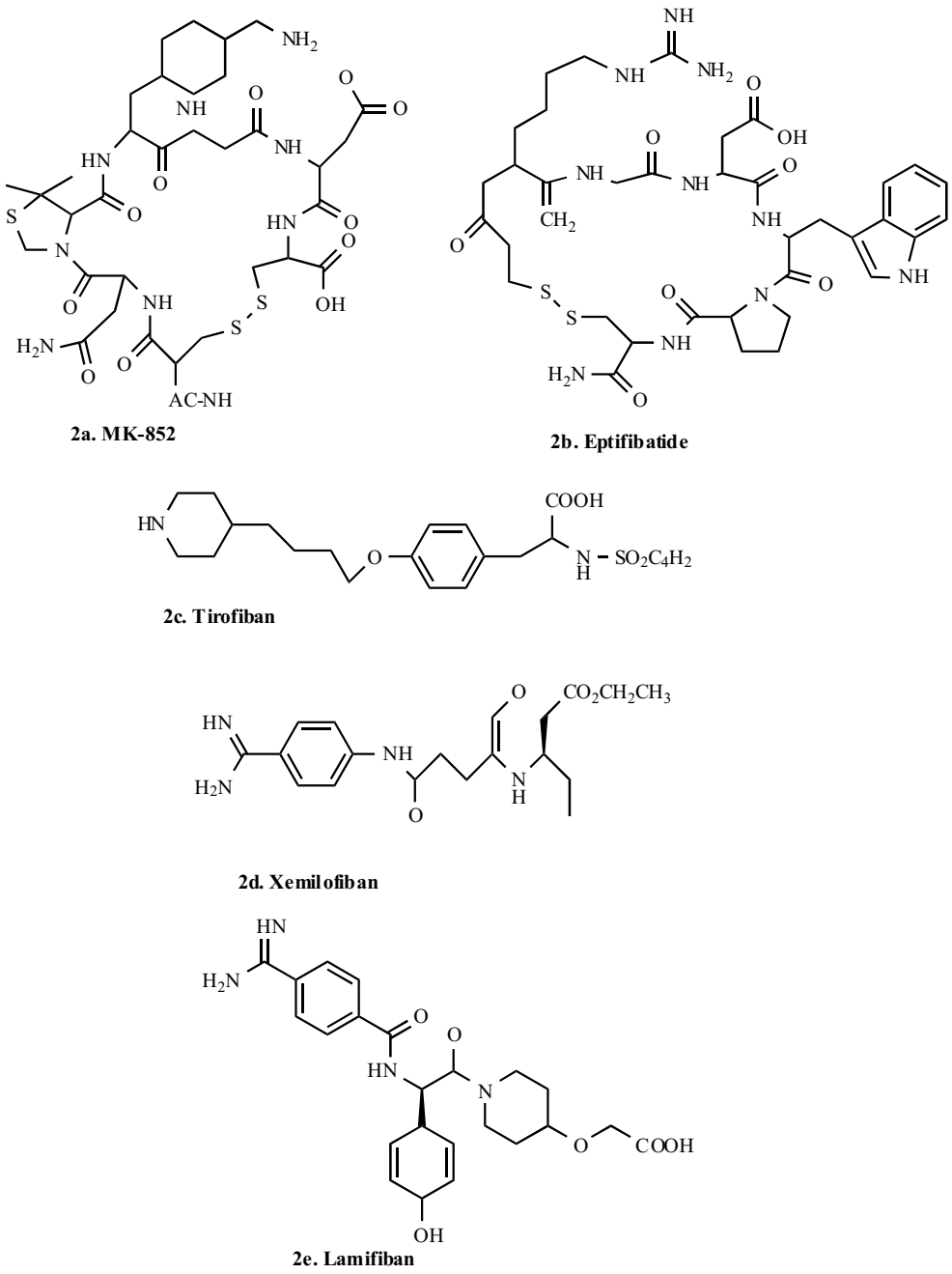

Fig. (2). Chemical structures of some GP IIb/IIIa inhibitors.

2a. MK-852 (cyclic RGD peptide)

2b. Eptifibatide (peptidie)

2c. Tirofiban (non peptide)

2d. Xemilofiban (orally active GP IIb/IIIa inhibitor)

2e. Lamifiban (peptide derivative)

AC denotes acetyl group.

Abciximab has been developed from a murine monoclonal antibody directed against the GP IIb/IIIa receptor [40]. The Fab fragments have been joined with the constant regions of human immunoglobulin, forming a chimeric compound**.** In order to prevent immunogenicity [41], the Fc fragment of each antibody (7E3) has been removed. It is produced by continuous perfusion in mammalian cell culture [8]. Abciximab engages the GP IIb/IIIa receptor, leading to a steric hindrance of the receptor (Fig. (**4**)). This is a different site from the ligand-binding RGD sequence [42]. Abciximab binds, with equal affinity, the beta$_3$ subunit and the avb$_3$ integrin (the vitronectin receptor), suggesting a role of the

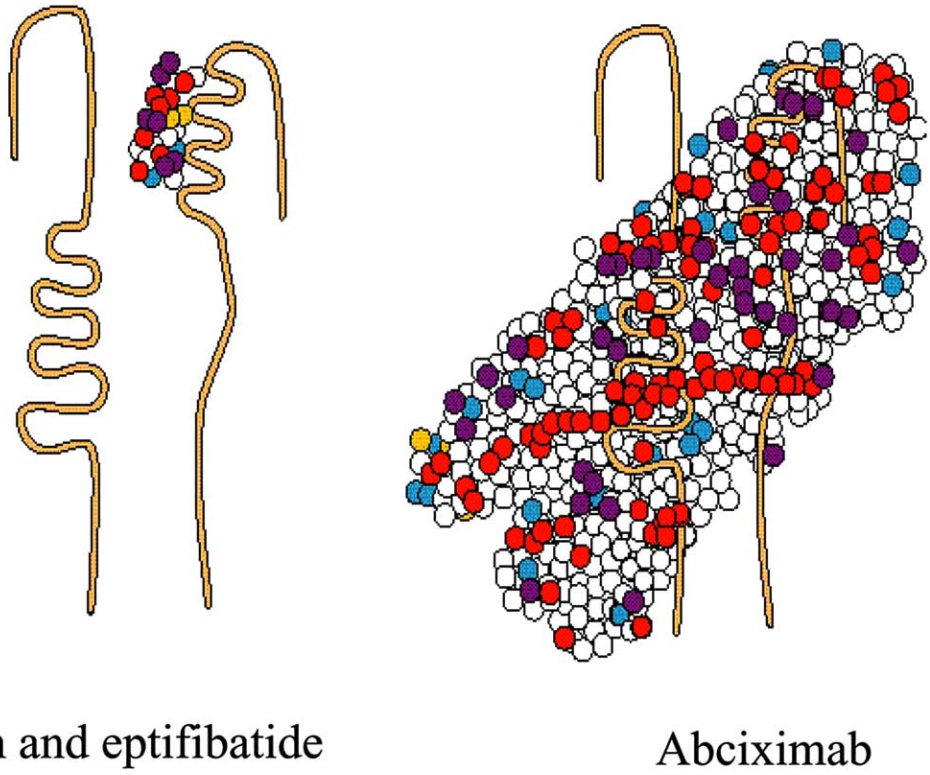

Fig. (3). Differences in binding specificity of small molecules eptifibatide and tirofiban (highly specific for GP IIb/IIIa) and abciximab (inhibitory effect by steric hindrance).

former and its conformational epitopes in ligand-receptor binding [35]. Furthermore, abciximab recognizes the activated white-cell integrin $\alpha_M\beta_2$ (Mac-1 or CD11b/CD18). In animal models of balloon angioplasty, $\alpha_v\beta_3$ (the vitronectin receptor) blockade can prevent smooth muscle cell (SMC) hyperplasia and $\alpha_M\beta_2$ inhibition can prevent stimulated monocyte-induced SMC apoptosis. Therefore, a role of abciximab in prevention of restenosis after angioplasty has been initially proposed, but not supported by data from clinical trials. Even if GP IIb/IIIa receptor is involved in thrombin formation, it is still not clear if abciximab is able to have direct antithrombin effects [43,44]. The antibody has unique pharmacokinetic properties, with the majority of the drug cleared from plasma within 25 min, but a slower clearance from the body with a catabolic half-life of up to 7 hours [23]. Nevertheless, remnant platelet-abciximab links can still be detected for more than 14 days after administration. This is mainly due to the high affinity of abciximab for the receptor. Its dissociation constant (K_d) from the GP IIb/IIIa receptor is 5 nmol/L [45]. Furthermore, the drug remains evenly distributed among the population of circulating platelets. With an average period of platelet circulation of about 7 days, it appears that abciximab can freely dissociate and reassociate with GP IIb/IIIa receptors as turnover of platelets in the circulation continues, thus prolonging the "biological" half-life of the drug.

Eptifibatide is a synthetic cyclic heptapeptide GP IIb/IIIa receptor antagonist, whose structure is based on that of barbourin, a peptide found in the venom of the pygmy rattlesnake *Sistrurus miliarius barbouri* . It contains the amino acid KGD sequence (Lys-Gly-Asp instead of Arg-Gly-Asp (RGD)) within a disulphide ring, which has highly specific anti GP IIb/IIIa properties [46] because it is an analog of the *C*-terminal sequence of the fibrinogen gamma chain [47,48]. Others changes in RGD sequences, as the replacement of the arginine group with an amidino- or benzamidino- containing group and the use of D-amino acids (i.e. lamifiban, Fig. (**2e**)), increases the resistance of these compounds to enzymatic degradation. Eptifibatide is 25% bound to plasma proteins and the dissociation constant (K_d) is 0.15 mcmol/L [46]. Mean peak plasma concentrations are reached five minutes after intravenous administration and a steady state is achieved in 4 to 6 hours [49]. Protein-binding is about 25%, leaving the remaining 75% to constitute the pool of pharmacologically active drug. Eptifibatide is up to 98% recovered in urine within 72 hours after discontinuation. Its elimination half-time is about 1.5 to 2.8 hours and the steady state volume of distribution is 0.23 L/Kg [50].

Tirofiban a non-peptide molecule whith dose- and concentration-dependent inhibition of platelet function by selective blockade of the GP IIb/IIIa receptor [51]. The non-peptide inhibitors do not have the (alpha)-amino acids characteristics of the peptide group. This drug, designed with the RGD sequence as the starting point, is a specific inhibitor of fibrinogen-platelet binding**.** No effects have been observed on Von Willebrand factor-platelet binding [52]. Approximately 65% of tirofiban molecules are bound to plasma proteins. An inhibition of 50% of the receptors is achieved with a drug concentration of 50 nmol/L (IC_{50}) with a inhibition constant (K_i) of 2.1 nmol/L [52]. Metabolism of Tirofiban in humans seems limited**,** with the majority of the drug eliminated unmodified in the urine. Like eptifibatide, its main route of excretion is renal, accounting for up to

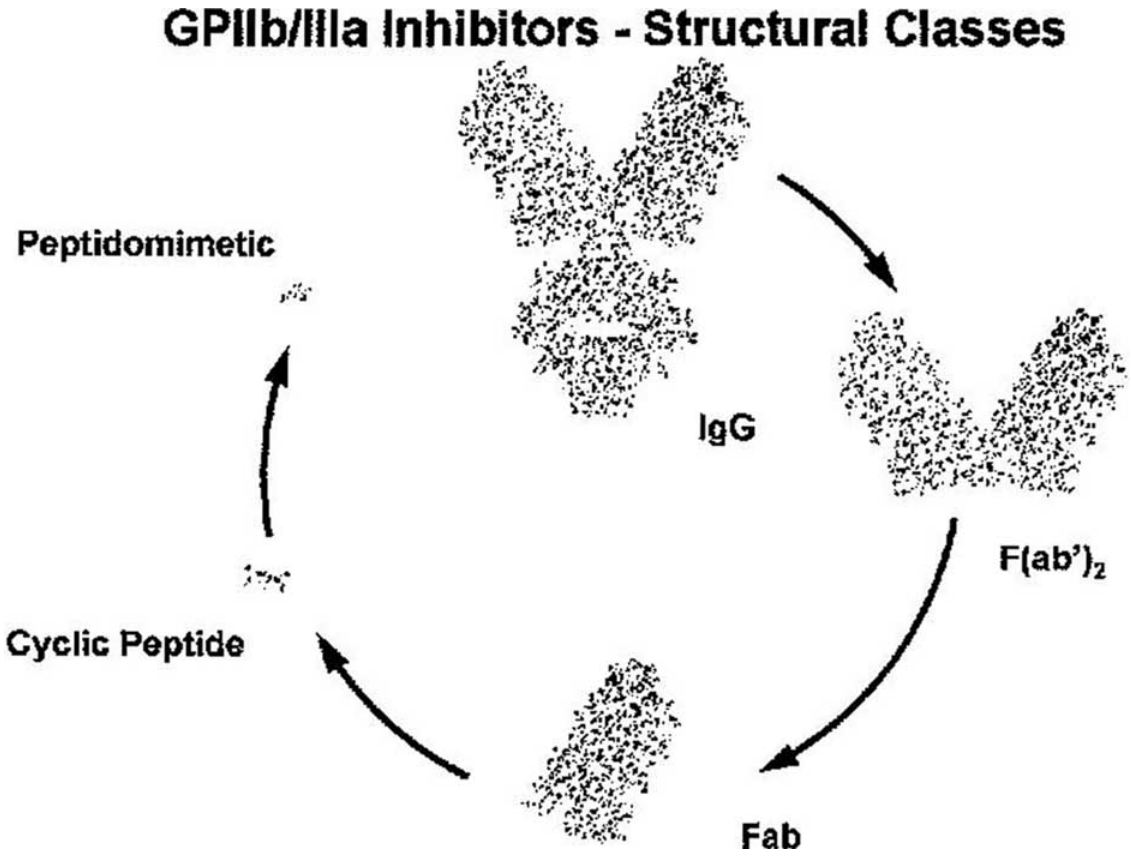

Fig. (4). Structural classes of GB IIb/IIIa inhibitors.

80% of total drug clearance**.** A small portion is eliminated in the gastrointestinal tract. Plasma clearance is reduced more than 50% in severe renal failure (creatinine clearance < 30 ml/min). Half-life of tirofiban is about 2 hours, with a steady state volume of distribution ranging from 21 L to 42 L [53]. Platelet function is restored in 8 hours after withdrawal of infusion.

Given the differences in pharmacodynamic and pharmacokinetic properties of these three drugs, effective dosages are also dissimilar. The amount of drug required to achieve at least 80% receptor occupancy depends on the dissociation constant. Drugs with high affinity (low dissociation constant, such as abciximab) are able to bind all the receptors at lower doses and for longer periods. Moreover, the effect of drugs with high affinity for the receptor may be easily overcome by platelet transfusions, since the concentration of unbound-active drug available for further receptor binding is very low. With low affinity compounds (high dissociation constant, such as eptifibatide and tirofiban) a higher dose is necessary to obtain the same receptor occupancy. As a result, a large amount of unbound drug will be detected in plasma at steady state.

Furthermore, with platelet activation the number of functionally active GP IIb/IIIa receptors changes and the antithrombotic efficacy of the drug is influenced by the ability to also block these "recruited" receptors. For this reason, drug administration consists of a bolus plus infusion strategy, which should guarantee homogeneous and

Table 1. Pharmacology Differences Between GP IIb/IIIa Inhibitors

	Abciximab	**Tirofiban**	**Eptifibatide**
Specificity			
$\alpha IIb\beta_2$	+++	+++	+++
$\alpha_v\beta_3$	+++	0	+
MAC-1	++	0	0
Duration of action	long	short	short
Induction in conformational change in GP IIb/IIIa	++	++	+
Inhibition of platelet mediated thrombin generation	+++	++	+

prolonged platelet inhibition until the drug is withdrawn. The infusion rate is also influenced by the dissociation constant of the drug, with high-affinity drugs requiring very low additional doses and low-affinity drugs larger doses.

Low-affinity drugs also have rapid clearance of the unbound plasmatic pool, causing achievement of accurate and sustained correct dosing more difficult. Unlike abciximab, with stronger affinity, small molecules are rapidly eliminated from the circulation once the infusion is stopped. The stoichiometry of both eptifibatide and tirofiban is more than 100 molecules of drug per GP IIb/IIIa receptor required to achieve full platelet inhibition. This compares with a stoichiometry of 1,5 molecules of abciximab per receptor. Therefore, they have the "biological" profile of short-acting agents whose effects on platelet aggregation rapidly dissipate (within 4 hours) once the drug infusion is completed [35]. On the other hand small molecules, because of their size, are not as likely as abciximab to induce an antibody response, albeit in general an extremely rare side effect .

Overall, the clinical benefit of these drugs is strictly linked to inhibition of platelet activation. This may explain their proven efficacy in prevention of adverse events after a PCI, when iatrogenic vessel injury stirs up a platelet "storm", and the lack of consistent results in acute coronary syndromes, whereby the role of platelets may vary according to the varied and complex pathogenetic factors [54,55]. In fact, in this last clinical setting beneficial effects have been observed in patients with diabetes [56] or positive for troponin I [57], subgroups possibly characterized by an exaggerated pro-thrombotic milieu.

Still**,** the pharmacology of GP IIb/IIIa inhibitors is only partially understood. More recent studies have pointed out their potential role as glycoprotein receptor agonists, able to transmit that outside-in signaling typical of platelet-fibrinogen binding [58,59]. This is thought to be more evident in presence of low plasma levels of the drug, leading to only moderate platelet inhibition, unable to overcome intrinsic agonist activity. Furthermore, low plasma levels of GP IIb/IIIa inhibitors seem to enhance, more than decrease, inflammation through the expression of platelet mediators of leukocyte-platelet aggregation [60].

In conclusion, even if beneficial effects have been demonstrated in several large clinical trials of GP IIb/IIIa inhibitors, the complexity of their interaction with the glycoprotein receptor requires further detailed studies to elucidate specific unique characteristics of single compounds.

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