Antiplatelet therapies: from aspirin to GPIIb/IIIa-receptor antagonists and beyond

Shaker A. Mousa

This review discusses recent advances in antiplatelet therapies, comparative analysis between the antiplatelet/antithrombotic efficacy of various antiplatelet strategies and that of platelet glycoprotein GPIIb/IIIa-receptor antagonists, issues in the development of chronic anti-GPIIb/IIIa-receptor therapy and potential adjunct strategies using GPIIb/IIIa-receptor antagonists. Acute coronary syndromes are secondary to unstable angina, ST-segment elevation, and acute myocardial infarction. These involve the rupture of a vulnerable atherosclerotic plaque, leading to platelet adhesion, activation and aggregation at the site of rupture. Several studies suggest that complex or ulcerated plaques, which might promote further thrombotic events, can persist for more than one month after the acute event. These data suggest the potential added benefit of chronic oral therapy with antiplatelet drugs beyond the well-documented benefit of acute intravenous use of various GPIIb/IIIa-receptor antagonists. However the efficacy–safety ratio or the risk–benefit ratio for chronic oral antiplatelet therapy needs to be defined. Both aspirin and clopidogrel are available for chronic oral use. By contrast, there are tremendous challenges ahead with the oral GPIIb/IIIa-receptor antagonists because of their lack of expected benefit over aspirin. However, much still remains to be defined with regard to the optimization of current and future antiplatelet therapies or their optimized combinations.

Atherosclerotic plaque disruption is the predominant pathogenic mechanism underlying the acute coronary syndromes. Plaque rupture leads to the exposure of collagen and von Willebrand factor (vWf) resulting in platelet adhesion, platelet activation, clotting activation and aggregation leading to occlusive thrombus formation. Although drugs that interfere with platelet activation and function have been available for years, more powerful agents with novel mechanisms of action are being developed, such as the glycoprotein GPIIb/IIIa-receptor antagonists. In acute myocardial infarction, aspirin significantly reduces cardiovascular mortality and reinfarction. Additionally, for secondary prevention, available data support the use of a platelet inhibitor. The role of antithrombotic therapy for the prevention or treatment of ischemic events in patients with coronary artery disease has stimulated enormous interest and is potentially beneficial for patients.

An ideal antiplatelet agent should selectively block thrombogenic platelet-dependent mechanisms in various vascular diseases, without interfering with the normal physiological platelet functions that are required in hemostasis and wound healing. Several antiplatelet strategies have already been developed and are under preclinical or clinical investigation.

Shaker A. Mousa, DuPont Pharmaceuticals Co., Wilmington, DE 19880, USA. tel: +1 302 695 8418, fax: +1 302 695 7407, e-mail: shaker.a.mousa@dupontpharma.com

 1359-6446/99/$ – see front matter © Elsevier Science Ltd. All rights reserved. PII: S1359-6446(99)01394-X

The role of platelets in vascular diseases

One of the early events following vascular injury is the adhesion of circulating platelets to exposed subendothelium[1,2]. This primary adhesion to the matrix activates the platelets, which subsequently secrete several different compounds[1], some of which attract more platelets to the lumen of the artery and promote aggregation. The aggregating platelets obstruct the artery, reducing blood flow to downstream tissues[1–3]. This can then lead to various vascular disorders, including cerebrovascular and cardiovascular ischemia, triggering strokes and myocardial infarction, respectively[1–5]. Importantly, this initially results in the formation of a highly cohesive thrombus, caused by platelet aggregation at the site of injury and the subsequent activation of the coagulation cascade on the platelet surface with the generation of thrombin, fibrinogen and fibrin formation. This enables the platelets to survive longer in the lumen of the artery within the fibrin network. It is quite clear from preclinical investigation of various arterial thrombosis models that if fibrin generation by antithrombin is modulated, then arterial thrombosis is inhibited. This is also true for the antiplatelet GPIIb/IIIa-receptor antagonists[6].

Box 1. Key milestones behind recent advances

- Platelets – Bizzizro, 1882
- Light transmittance aggregometry – Born, 1962
- Aspirin – antiplatelet trials – 1960s–present
- Ticlpidine – late 1980s; clopidogrel – 1990s (ADP inhibitors)
- Platelet GPIIb/IIIa integrin – 1980s
- GPIIb/IIIa monoclonal antibody – B. Coller, 1980s
- RGD mimetics (cyclic peptides, peptidomimetics, non-peptides) – 1980s
- Clinical trials intravenous/oral – 1990s

When platelets are activated and degranulated, they secrete various biologically active mediators and substrates[7,8]. The alpha granules contain proteins such as fibrinogen, plasminogen activator inhibitor 1 (PAI-1) and growth factors, whilst the dense granules contain serotonin, ATP and many other small molecules. These mediators can amplify the signal and cause serious complications. Growth factors help recruit smooth muscle cells to the vascular lesion and PAI-1 increases resistance to thrombolysis.

The role of platelets in various thrombotic vascular diseases is shown in Fig. 1. There are different levels of cardiovascular complications depending on the extent of arterial occlusion. These include unstable angina (transient ischemic attack), which is a relatively mild complication, and acute myocardial infarction (stroke), which is a severe complication. The latter event can happen after either spontaneous plaque rupture or mechanical intervention[1–5]. Some of the key milestones underlying the recent advances in antiplatelet therapies are shown in Box 1. Figure 2 is a cross-section of the coronary artery from a patient who died from a myocardial infarction and shows an occlusive thrombus in the lumen. Scanning electron microscopy demonstrates that the thrombus in the coronary artery post-myocardial infarction is rich in platelets together with fibrin strands (Fig. 3).

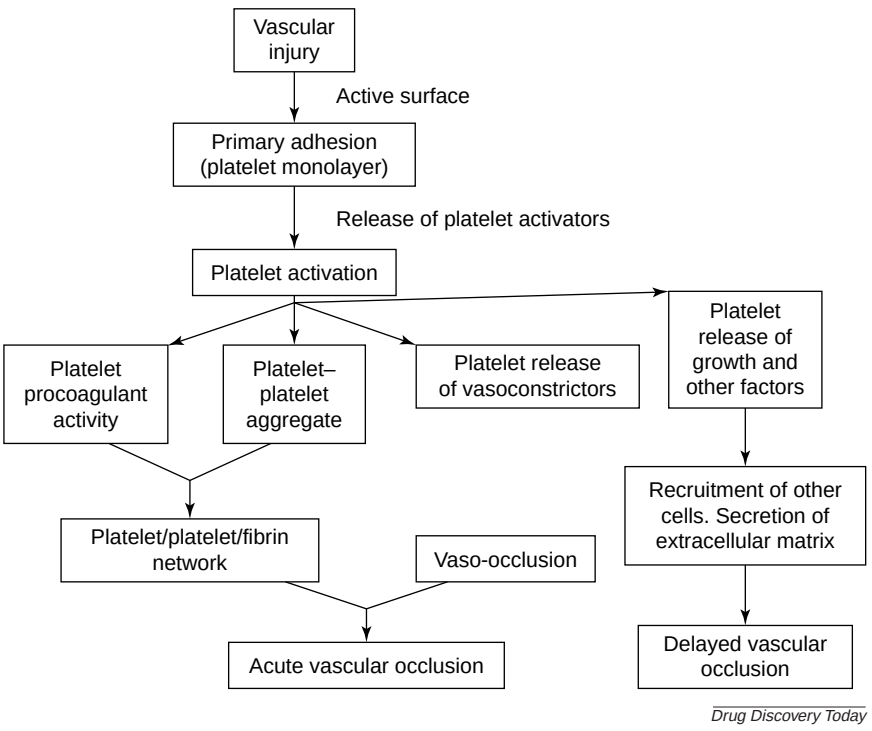


Figure 1. *Diagrammatic sketch illustrating the different processes and mediators involved in acute and delayed vascular occlusion.*

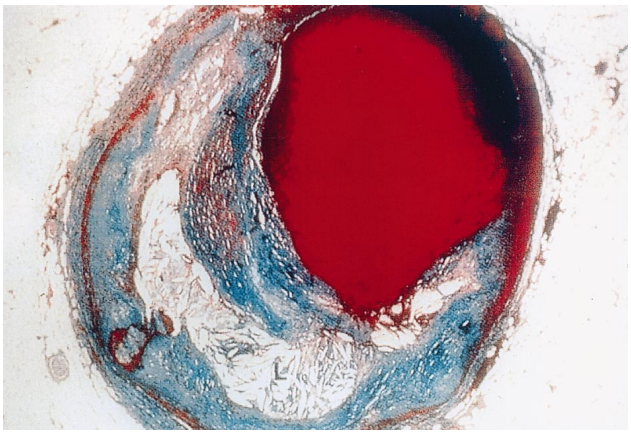


Figure 2. *Photomicrograph showing a stained coronary artery section from a patient that died of post-myocardial infarction. This section illustrates the point of plaque rupture and the formation of totally occlusive coronary artery thrombus.*

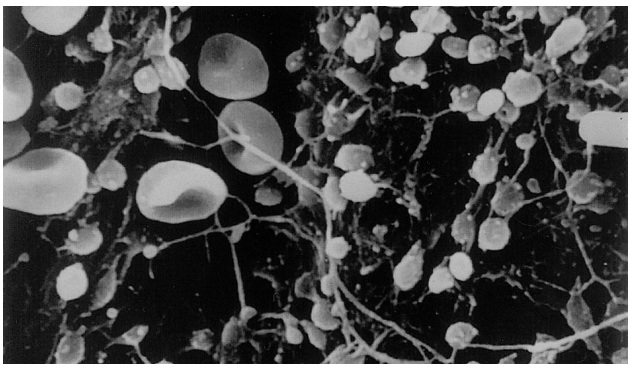


Figure 3. *A scanning electron micrograph (at a magnification of ×1250) demonstrating the composition of the thrombus in the coronary artery post-myocardial infarction. The thrombus is shown to be platelet-rich with some fibrin and white blood cells.*

The ideal antiplatelet agent

An ideal antiplatelet agent should specifically block thrombogenic platelet-dependent mechanisms in various vascular diseases, without interfering with the normal platelet functions that are required in hemostasis and wound healing. Additionally, the ideal agent should not have any major adverse effects, should be suitable for various routes of administration (e.g. intravenous, oral), and the duration of action should fulfill the required therapeutic efficacy. Several antiplatelet strategies have already been developed or are under preclinical or clinical investigation (Table 1). None of the available antiplatelet compounds meets all of these criteria.

Table 1. Agents modulating platelet function

Pathways	Agents
Platelet adhesion	
von Willebrand factor	Glycoprotein GPIb-receptor antibody
	Antibody to von Willebrand factor
	Inactive von Willebrand factor fragments
Platelet aggregation	
Cyclooxygenase pathway	
• Cyclooxygenase	Aspirin
• Thromboxane A_2 synthase	Dazoxiban
• Thromboxane A_2 receptor	Thromboxane A_2-receptor blocker
• Thromboxane synthase and receptor	Ridogrel
ADP	Ticlodipine, clopidogrel, P_{2T}-receptor antagonists
Thrombin	Hirudin, hirulag, argatroban, small-molecule inhibitors, thrombin-receptor antagonists
Serotonin	Ketanserin
Platelet-activating factor	Platelet-activating factor antagonists
Phosphodiesterase	Dipyridamole, caffeine
Glycoprotein GPIIb/IIIa receptor	RGD-mimetics (intravenous and oral)

Chronic therapy with antiplatelet drugs in ischemic diseases

Antiplatelet agents and stroke

The benefit of aspirin in improving vascular outcome is well established. It reduces the relative risk for stroke, myocardial infarction and vascular death by approximately 25% compared with placebo[9–12]. It has been previously shown that ticlopidine is more effective than aspirin (about 12% relative risk reduction for stroke or death)[13]. However, ticlopidine has several adverse effects. In 1996, the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial showed that clopidogrel, a new thienopyridine similar to ticlopidine, is also slightly more effective than aspirin, whilst being as safe[13]. Clopidogrel showed a very modest benefit over aspirin (absolute reduction of 0.5% and relative risk reduction of 8.7% compared with aspirin). The European Stroke Prevention Study 2 (ESPS-2) also showed in 1996 that dipyridamole alone

prevents strokes and that when combined with aspirin, is more effective and probably comparable to ticlopidine and clopidogrel[13]. Dipyridamole combined with aspirin modestly (by approximately 13%) reduced the relative risk of stroke or death compared with aspirin alone. The Chinese Acute Stroke Trial (CAST) and the International Stroke Trial (IST) demonstrated that aspirin given at the time of an acute ischemic stroke reduces the risk for early death (about five fewer per 1000 treated), recurrence and death (about ten fewer per 1000 treated)[14]. Overall, the benefits of aspirin in acute stroke treatment and stroke prevention are definite but modest.

Antiplatelet agents and ischemic heart disease

Aspirin is an established therapy for the management of acute myocardial infarction, unstable angina and secondary prevention of myocardial infarction. It inhibits cyclooxygenase, which is a key enzyme in the biosynthetic pathway leading to the production of thromboxane A_2. Hence, it only inhibits one of the many activation pathways leading to platelet aggregation. Other antiplatelet agents that have been evaluated in clinical trials include ticlopidine and clopidogrel. They inhibit ADP-mediated platelet aggregation, but again, these agents are known to be effective against only one of the many agonists[1,15,16]. The final common pathway for platelet aggregation involves binding of the GPIIb/IIIa receptor to fibrinogen. Several inhibitors of the GPIIb/IIIa receptor have been developed and shown to have an important role as adjunctive therapy in angioplasty[17]. Recent trials have been performed in patients with unstable angina, and trials of adjunctive therapy are currently under way both in patients receiving thrombolysis for acute myocardial infarction and for secondary prevention[18–20]. These drugs have several features, including specificity for blockade of the GPIIb/IIIa receptor, half-life, duration of effect, route of administration and chemical nature (Box 2)[6]. Recently concluded and ongoing trials of both intravenous and oral agents are expected to provide further support for the introduction of these agents into the clinical management of patients with acute coronary syndromes.

Clopidogrel was compared with aspirin in the CAPRIE international study in over 19,000 patients with symptomatic atherosclerotic disease, as manifested by recent ischemic stroke or myocardial infarction, or by established peripheral vascular disease. Clopidogrel 75 mg provided a significant 8.7% relative risk reduction over aspirin for the outcome cluster of stroke, myocardial infarction and vascular death and reduced the risk of ischemic events of every kind in the overall CAPRIE population, the greatest risk reduction being for the incidence of myocardial infarction[13]. Hematological tolerance was satisfactory and clopidogrel had a better gastric tolerability profile than aspirin. Given this favorable efficacy–safety ratio, clopidogrel represents a new advance in the management of atherothrombotic disease, as it appears to inhibit platelet aggregation induced mainly by ADP. It is likely that the combination of clopidogrel with thromboxane-dependent platelet activation inhibitors, such as aspirin, might be advantageous. Trials of combined therapy with clopidogrel and aspirin are under way.

Box 2. Discovery of different classes of platelet GPIIb/IIIa antagonists

Chemical nature
- Monoclonal antibody – c7E3, YM337
- RGD, cyclic analogs–Integrilin, DMP728
- Non-peptide – tirofiban, lamifiban, xemilofiban, lefradafiban, roxifiban, lotrafiban

Route of administration
- Intravenous – c7E3, integrilin, tirofiban, lamifiban
- Oral – xemilofiban, lefradafiban, DMP754
- Other routes: intranasal – DMP755

Binding kinetics
- Selectivity for activated versus resting platelets – DMP728 versus c7E3, XV454, DMP802

Platelet dissociation rate (duration of action)
- Fast – integrilin, tirofiban, lamifiban, orbofiban
- Intermediate – DMP754 (roxifiban)
- Slow – c7E3, DMP802, XV454

GPIIb/IIIa-receptor blockade and platelet functions

It is very important to point out that blockade of platelet GPIIb/IIIa receptors does not interfere with changes in platelet shape or secretory activity[21,22]. Platelet GPIIb/IIIa-receptor antagonists inhibit platelet–platelet binding but not binding of platelets to collagen or vWf. Platelets obtained from Glanzmann thrombasthenia have dysfunctional GPIIb/IIIa receptors, impaired platelet aggregation and bind to fibrinogen in response to various platelet activators, but have a normal secretory function in response to platelet agonists[22]. However, upon blockade of GPIIb/IIIa receptors with certain GPIIb/IIIa-receptor antagonists, there is a shift in the threshold of the agonist concentration required to attain the same level of secretion compared with untreated platelets. It is also important to highlight that, when platelets are inhibited using

GPIIb/IIIa-receptor antagonists, the size of the platelet mass in the lumen and the thrombus size are markedly decreased[23], and hence the quantity of platelet secretion is reduced.

There are 60,000–90,000 fibrinogen-binding sites per platelet[24–26]. Fibrinogen bound to platelets through GPIIb/IIIa receptors serve to crosslink two or more platelets. Thus, blocking this receptor inhibits platelet aggregation and fibrinogen binding induced by any of the agonists or even their combinations[6].

It is also crucial to consider the potency of the agonist and plasma-free Ca^{2+} levels, which can be affected by certain anticoagulants such as citrate. Generally, *ex vivo* or *in vitro* platelet aggregation is measured using 10 μM ADP in platelet-rich plasma obtained from citrated blood. This concentration of ADP only produces partial activation or partial expression of the GPIIb/IIIa receptors, but other agonists such as thrombin, TRAP or collagen induce maximal activation of the GPIIb/IIIa receptors. Consequently, a disparity between inhibition of platelet aggregation and bleeding time extension can be demonstrated when using a weak platelet agonist at relatively low concentrations. It is important to consider carefully the agonist, its concentration and plasma-free Ca^{2+} levels.

Platelet procoagulant activity

Platelet activation with subsequent aggregate formation generates a phospholipid surface that can activate and support the procoagulant activity with the subsequent generation of thrombin. Prothrombinase complexes are assembled on phospholipid surfaces and involve Ca^{2+}-dependent stoichiometric association of cofactors Va and Xa. Blockade of GPIIb/IIIa receptors attenuates this platelet procoagulant activity, thereby attenuating fibrin formation[27]. However, further research is needed to elucidate this mechanism.

Classes of GPIIb/IIIa-receptor antagonists

Several classes of GPIIb/IIIa-receptor inhibitors, including monoclonal antibodies [most notably murine or chimeric anti-b3 (c7E3 Fab) such as abciximab (ReoPro)], RGD peptides, cyclic compounds (DMP728, integrilin), lamifiban (Ro449883, a pseudopeptide) and tirofiban (MK383, a non-peptide platelet GPIIb/IIIa-receptor antagonist), are either already on the market or in an advanced stage of development. Platelet GPIIb/IIIa-receptor antagonists can be classified (see Box 2) on the basis of chemical nature (monoclonal antibody, linear peptide, cyclic peptide, peptidomimetic, non-peptide), route of administration (intravenous or oral), binding-kinetic profiles (K_d) to activated versus unactivated platelets, and differences in the dissociation rate constant (K_{off}). A key feature of all small-molecule GPIIb/IIIa-receptor antagonists is the presence of an anionic carboxy-terminal (CO_2^-) separated by a spatial chemical moiety and a certain distance from the cationic basic amino-terminal (benzamidine, piperidine, guanidine). The distance between the anionic and cationic terminals is crucial for optimal binding affinity and specificity for the platelet GPIIb/IIIa receptors with minimal to no cross-reactivity with other closely related RGD-dependent integrins such as $\alpha_v\beta_3$, $\alpha_v\beta_5$ or $\alpha_5\beta_1$.

Two classes of GPIIb/IIIa-receptor antagonists are recognized. Class I compounds (e.g. roxifiban, DMP802, XV454) bind to both forms of GPIIb/IIIa receptors (resting and activated platelets) with comparable K_d values and relatively slow platelet dissociation rates. Class II compounds (e.g. L734217, MK852, DMP728) bind with much higher affinity to the activated form of GPIIb/IIIa than to the resting form and have relatively fast platelet dissociation rates [for example, for selective antagonists such as L734217, K_d(activated] = 5 nM and K_d(resting) = 620 nM)[28]. Both classes can effectively inhibit platelet aggregation *ex vivo*. The potential clinical advantages or disadvantages of selective (Class II) versus nonselective (Class I) GPIIb/IIIa-receptor antagonists remain to be defined in clinical trials.

Comparative antiplatelet effects between GPIIb/IIIa-receptor inhibitors and other antiplatelet agents

The prototype of the GPIIb/IIIa-receptor antagonists, DMP728 [cyclo(D-2-aminobutyrate-*N*-methyl-L-arginyl-glycyl-L-aspartyl) 3-aminomethylbenzoic acid], which was one of the first molecules discovered at DuPont (Wilmington, DE, USA), will be used to illustrate the pharmacology of GPIIb/IIIa-receptor antagonists[29,30]. DMP728, like many other small-molecule RGD-mimetics, is selective for activated platelets over resting platelets. Experiments were carried out to compare the antiplatelet effects of DMP728 with those of other antiplatelet agents using a light-transmittance platelet aggregation assay. In this assay, there is an initial shape change in response to certain agonists, and then an increase in light transmittance as the platelets aggregate. By contrast, platelet GPIIb/IIIa-receptor antagonists have no effect on the shape changes, but they do block platelet aggregation.

Direct thrombin inhibitors are selective for thrombin-induced platelet aggregation over aggregation induced by other agonists[15]. Up to 650 mg aspirin produces a long-lasting antiplatelet effect against arachidonic acid and, to a lesser extent (40–60%), against ADP (Ref. 16). DMP728 was

found to inhibit platelet aggregation induced by any individual platelet aggregating agent, or even their combination, with an IC_{50} ranging from 20 to 50 nM (Ref. 16). In humans, the dose–response curve for the inhibition of *ex vivo* platelet aggregation by DMP728 in relation to the plasma level is similar to the dose–response curve of inhibition of platelet aggregation *in vitro*. In summary, the anti-aggregatory efficacy of different antiplatelet agents against various platelet agonists demonstrated the following:

- Aspirin inhibits platelet aggregation in the micromolar range (IC_{50} = 5–10 μM) against arachidonic acid
- Ticlopidine or clopidogrel are more effective against ADP and collagen than other agonists
- Direct thrombin inhibitors are very effective against thrombin
- GPIIb/IIIa-receptor antagonists are effective against all agonists.

Direct thrombin inhibitors, such as hirudin and PPACK (D-Phe-Pro-Arg-chloromethylketone), inhibit platelet PAI-1 secretion induced by thrombin, whereas platelet GPIIb/IIIa-receptor antagonists have no effect. It is very important to note that, despite the lack of effect of GPIIb/IIIa-receptor antagonists on platelet secretion, a reduced platelet mass in the artery lumen will reduce net platelet secretion in the region of the thrombus.

In determining the effects of platelet GPIIb/IIIa-receptor blockade on the degranulation of alpha granules, a flow-cytometric study with different platelet GPIIb/IIIa-receptor antagonists versus direct thrombin inhibitors was performed using a double-fluorescent label for P-selectin and another for the GPIIb/IIIa receptor. This study showed that thrombin activation of the platelets increased the fluorescence intensity of P-selectin and GPIIb/IIIa-receptor expression. Thrombin inhibitors such as hirudin abolished the increased fluorescence of both P-selectin and GPIIb/IIIa-receptor fluorescent labels induced by thrombin. By contrast, GPIIb/IIIa-receptor antagonists inhibit only the fluorescence shift of the GPIIb/IIIa-receptor label, but not the P-selectin label[15]. A summary of the antiplatelet profiles of high-sffinity platelet GPIIb/IIIa-receptor antagonists is shown in Box 3.

Comparative antithrombotic efficacy

In the Folts canine arterial thrombosis model, the platelet GPIIb/IIIa-receptor antagonist DMP728 given intravenously at 5 and 10 μg kg^{-1} resulted in 50% and 100% inhibition, respectively, of ADP-induced aggregation and 100% inhibition of cyclic flow reduction at either dose[23]. Even upon rechallenge with epinephrine (adrenaline), the antithrombotic benefit of the GPIIb/IIIa-receptor antagonist was not affected. However, with aspirin which, in the same model, works about 40% of the time, rechallenge with epinephrine eliminated the marginal antithrombotic effects of aspirin with the subsequent reccurrence of oscillatory cyclic flow reductions. This is also the case with direct thrombin inhibitors upon rechallenge with epinephrine in this model. These data indicate the unique advantage of GPIIb/IIIa-receptor antagonists as a potent antithrombotic strategy compared with other antiplatelet strategies.

The effects of the direct thrombin inhibitor hirudin and the platelet GPIIb/IIIa-receptor antagonists 7E3 or DMP728 on the prevention of thrombosis and rethrombosis after thrombolysis in a chronic canine coronary artery thrombosis model demonstrated superior antithrombotic efficacy of the platelet GPIIb/IIIa-receptor antagonists[31,32]. The universal anti-aggregatory efficacy of the GPIIb/IIIa-receptor antagonists and efficacy of the thrombin inhibitors and aspirin against thrombin- or arachidonic acid-induced platelet secretion of growth factors or PAI-1 suggest the potential efficacy and safety benefits of their combination in various thrombotic disorders and pharmacological and mechanical coronary intervention procedures.

The new class of GPIIb/IIIa-receptor antagonists combined with aspirin and heparin were shown to reduce ischemic events in high- and low-risk patients with stents, unstable angina and non-Q-wave infarction, as well as during coronary interventions. Abciximab (ReoPro, c7E3 Fab) and related antagonists of platelet GPIIb/IIIa receptors were the most potent inhibitors of platelet aggregation. Currently, their use is restricted to acute, severe platelet ischemic

Box 3. Antiplatelet profiles of high-affinity platelet GPIIb/IIIa-receptor antagonists

- Inhibit platelet aggregation against all known agonists
- Inhibit fibrinogen binding to activated platelets regardless of the agonist
- Inhibit clot retraction
- Attenuate platelet procoagulant activities
- Deaggregatory and lytic efficacy
- Bind to activated and unactivated platelets
- Do not affect GPIb-mediated primary adhesion under shear
- Do not interfere with platelet secretory function in fluid phase but interfere when platelets adhere to vWf

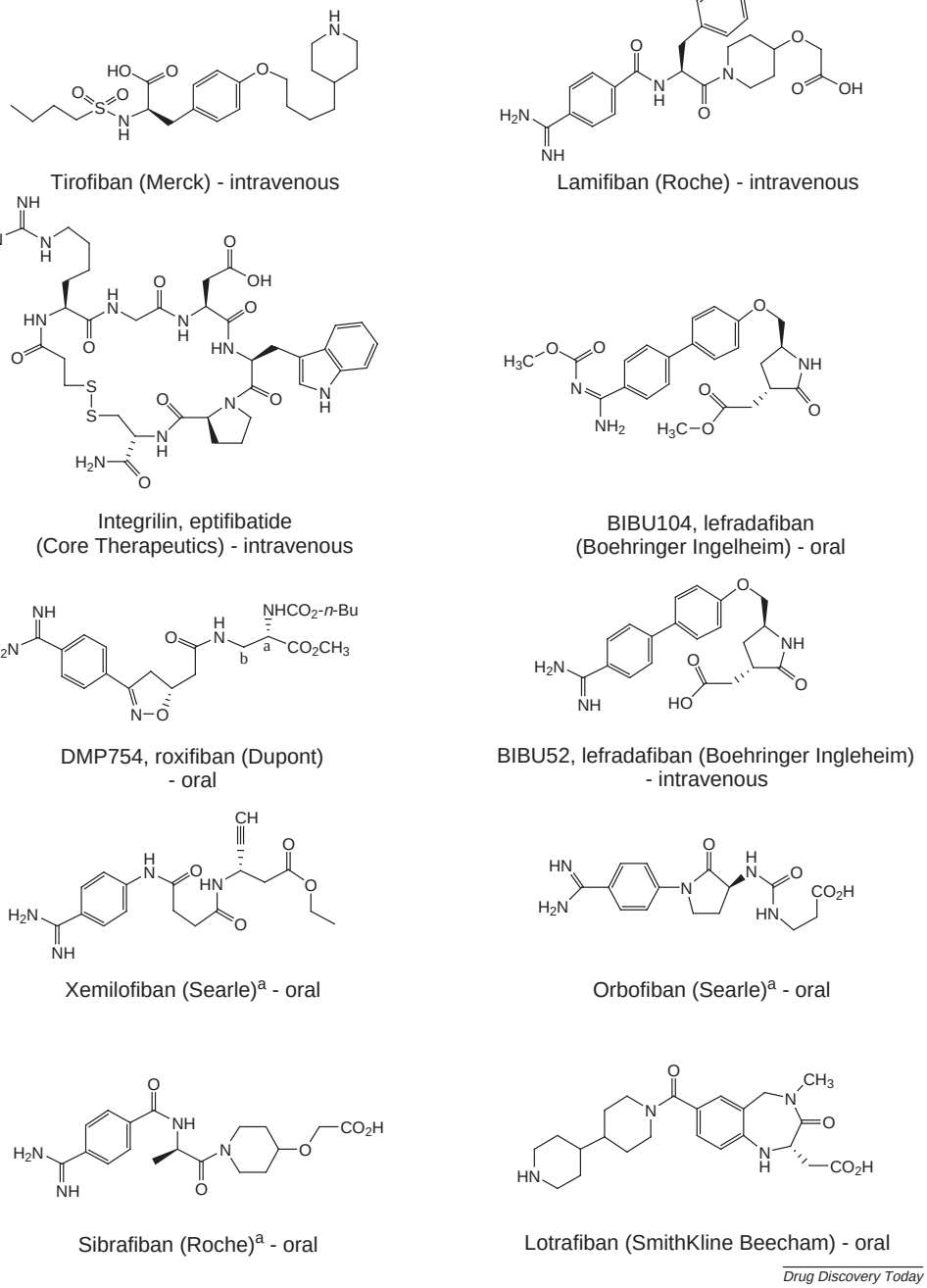


Figure 4. *Structure of selected glycoprotein GPIIb/IIIa-receptor antagonists approved for clinical use or in clinical development. [a]Oral xemilofiban, orbofiban and sibrafiban have recently been dropped from clinical development. The free acid form of orbofiban, sibrafiban and lotrafiban is shown. All oral platelet GpIIb/IIIa-receptor antagonists are monoprodrug esters, except sibrafiban and lefradafiban.*

syndromes such as unstable angina pectoris. Several peptidomimetics and non-peptide analogs are currently under clinical investigation for intravenous and oral use (Fig. 4).

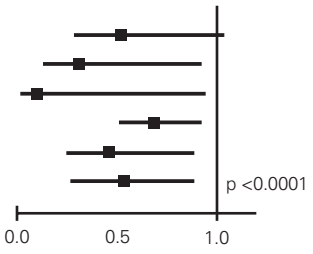
Acute therapy with platelet GPIIb/IIIa-receptor antagonists

The GPIIb/IIIa-receptor antagonists that have been studied include abciximab (a murine monoclonal antibody), integrilin (a synthetic peptide) and tirofiban, lamifiban, xemilofiban, sibrafiban and lefradafiban (synthetic non-peptides). The majority of clinical trials of GPIIb/IIIa-receptor antagonists have been performed in patients with unstable angina or acute myocardial infarction and in patients undergoing percutaneous coronary interventions in which an intracoronary thrombus might lead to ischemic complications. There is abundant evidence that GPIIb/IIIa-receptor antagonists reduce the risk of death, acute myocardial infarction and urgent revascularization procedures in high- and low-risk patients undergoing percutaneous coronary interventions[17,33]. Abciximab remains the most studied of these agents in interventional settings. Data are accumulating on synthetic peptide and non-peptide GPIIb/IIIa-receptor antagonists that also demonstrate lower rates of death and ischemic complications in the treatment of acute coronary syndromes. In patients that have had a successful response to intravenous GPIIb/IIIa-receptor antagonists, oral agents could represent an option for secondary prevention. Additional studies are required to determine further uses for these agents.

The use of platelet GPIIb/IIIa-receptor inhibition in cardiology began in 1994 with the publication of the results

Table 2. Effect of intravenous glycoprotein (GP)IIb/IIIa-receptor antagonists on composite event rates and percentage in unstable angina patients

Antagonist	Treatment Event rate	(%)	Placebo Event rate	(%)	Relative risk (95% CI)
c7E3	9 in 30	30.0	16 in 30	53.3	
Integrilin	5 in 56	8.9	13 in 51	25.5	
Tirofiban	1 in 71	1.4	4 in 31	12.9	
Lamifiban	68 in 242	28.1	51 in 123	41.5	
c7E3 (EPIC)	19 in 313	6.1	20 in 156	12.8	
Total	109 in 789	13.8	110 in 438	25.1	



Glycoprotein IIb/IIIa-receptor antagonism in unstable angina. Results of pilot studies showing the antagonist used, the event rates in the treated and control groups, the relative risks and 95% confidence interval and the p values. The end points were death, myocardial infarction and refractory ischemia.

of the Evaluation of 7E3 for the Prevention of Ischemic Complications (EPIC) trial[17]. EPIC demonstrated that the GPIIb/IIIa-receptor blocker abciximab, administered as a bolus and a 12-h infusion, afforded protection against ischemic complications in high-risk patients undergoing angioplasty and atherectomy, including those with unstable angina or evolving myocardial infarction. A significant reduction in the incidence of death, acute myocardial infarction or revascularization was apparent at 30 days and also sustained at six-month and three-year follow-up. The subsequent Evaluation in PTCA to Improve Long-Term Outcome with Abciximab GPIIb/IIIa Blockade (EPILOG) study extended these findings to the full spectrum of coronary intervention patients, and confirmed that abciximab provides similar benefits in low-risk patients[17,33]. The EPILOG trial also demonstrated that any excess bleeding risk associated with potent antiplatelet therapy could be reduced to placebo levels through the use of a low-dose, weight-adjusted heparin regimen, early vascular sheath removal and elimination of routine postprocedural heparinization. A similar pattern of benefit has emerged from clinical trials of other GPIIb/IIIa-receptor inhibitors such as integrilin, lamifiban and tirofiban (Table 2). Trials are currently under way to clarify the benefits of GPIIb/IIIa-receptor blockers in patients undergoing stenting and as an adjunct to thrombolytic therapy or primary angioplasty in patients with acute myocardial infarction.

Intravenous administration of GPIIb/IIIa-receptor inhibitors provides improved benefits in addition to those of aspirin and heparin in the acute management of patients with unstable angina and in those undergoing angioplasty (Table 2). Efficacy is the primary concern in the acute setting, but the incidence of side-effects and the consequent impact on compliance becomes of greater concern during the chronic oral administration of these compounds because of the steep dose–response relationship. Newer compounds under consideration for chronic administration as platelet inhibitors and antithrombotic drugs include GPIb-receptor inhibitors and inhibitors of tissue factor/VIIa and factor Xa.

Abciximab: pharmacology and extended benefits

The clinical utility of abciximab (ReoPro, c7E3 Fab) is based on several trials involving coronary artery intervention procedures: EPIC (high-risk abrupt closure), EPILOG (broad entry criteria), CAPTURE (refractory unstable angina), EPISTENT (stent eligible) and RAPPORT (direct angioplasty)[17,34,35]. The potent, rapid and sustained blockade of platelet GPIIb/IIIa receptors and perhaps its blockade by $\alpha_v\beta_3$ might be key to the dramatic early antithrombotic benefits. Early benefits were maintained for over three years in patients receiving 12-h abciximab treatment in the EPIC trial. These unique pharmacological characteristics might also provide benefits in other disabling thrombotic conditions such as stroke, unstable angina and acute myocardial infarction.

Intravenously administered small-molecule GPIIb/IIIa-receptor antagonists

Integrilin is a cyclic heptapeptide KGD analog and IMPACT II (coronary intervention, broad entry criteria) and PURSUIT (unstable angina, chest pain <24 h, ischemic ECG changes) both demonstrated significant clinical benefits[18]. Meanwhile, clinical benefits of tirofiban have been demonstrated in RESTORE (coronary intervention, high risk of abrupt closure assessed by clinical and anatomical criteria), PRISM and PRISM PLUS (unstable angina, chest pain at 12 h and 24 h)[19]. Furthermore, PARAGON (unstable angina, chest pain <12 h, ECG changes) demonstrated significant clinical benefits of lamifiban[20], although similar studies in Canada were stopped because of a lack of efficacy and nuisance bleeding.

Chronic therapy with oral platelet GPIIb/IIIa-receptor antagonists

A high level of platelet antagonism has been required when GPIIb/IIIa-receptor antagonists have been employed

as acute therapy of coronary arterial disease. However, the requirements for chronic therapy using orally active agents are only now being determined. Interaction with aspirin and other antiplatelet and anticoagulant drugs leads to shifts in the dose–response curves for both efficacy and unwanted side-effects, such as increased bleeding time. As experience with this new class of agents is gained, the benefits and pitfalls associated with their use will become clearer.

SC5468A (xemilofiban)

The active metabolite of xemilofiban, SC54701A, is a potent selective inhibitor of GPIIb/IIIa receptors compared with other integrins. More than 50% of the orally administered prodrug was absorbed in dogs and half that amount was converted to the active agent[36]. Platelet aggregation was abolished for more than 8 h after a single oral dose of 2.5 mg kg^{-1}. After intravenous administration in dogs, the elimination half-time of the active moiety was 6.5 h (4.7 ± 0.1 h) with a total plasma clearance of 0.3 l h^{-1} kg^{-1}. The results of a dose-ranging study show that oral administration of the prodrug produces a dose-dependent inhibition of platelet aggregation, which is maintained during a 14-day administration period in dogs, with no adverse effects. At a dose that inhibited collagen-induced aggregation by 80%, bleeding time was increased 2.5-fold. Whether these results will translate into less bleeding in a clinical situation upon long-term administration remains to be determined.

Sibrafiban

Sibrafiban (Ro483657) is an oral selective GPIIb/IIIa-receptor antagonist. The Thrombolysis in Myocardial Infarction (TIMI)-12 trial was a Phase II, double-blind dose-ranging trial designed to evaluate the pharmacokinetics (PK), pharmacodynamics (PD), safety and tolerability of sibrafiban in 329 patients after acute coronary syndromes[37]. In the PK/PD cohort of TIMI-12, 106 patients were randomized to receive one of seven dosing regimens of sibrafiban, ranging from 5 mg daily to 10 mg twice daily for 28 days. In the safety cohort, 223 patients were randomized to one of four dose regimens of sibrafiban (ranging from 5 mg twice daily to 15 mg once daily) or aspirin for 28 days. High levels of platelet inhibition were achieved, with mean peak values of 47–97% inhibition of 20 μmol l^{-1} ADP-induced platelet aggregation on day 28 across all seven doses. Twice-daily dosing provided more sustained platelet inhibition (mean inhibition of 36–86% on day 28). Recently, the SYMPHONY trial demonstrated no significant difference between the sibrafiban- and the aspirin-treated group[38].

Lefradafiban (BIBU104)

Lefradafiban (BIBU104) is an orally active prodrug of the free acid active form, BIBU52. Escalating single and multiple oral doses between 10 and 100 mg have been investigated in human volunteers and found to be well tolerated[39].

Roxifiban

DMP754 (roxifiban) is an orally active GPIIb/IIIa-receptor antagonist for the prevention and treatment of thromboembolic disorders. *In vivo* studies in various animal models showed that oral doses of DMP754 (0.3 mg kg^{-1}) produced a >60% inhibition of *ex vivo* platelet aggregation with potent antithrombotic efficacy[40]. The activity of roxifiban (DMP754) resides in its carboxylic acid metabolite (known as XV459).

Lotrafiban

SB214857 (lotrafiban) is an orally active antiplatelet drug and a potent and specific GPIIb/IIIa-receptor inhibitor. It demonstrated dose-dependent inhibition of *ex vivo* platelet aggregation in man at doses ranging from 5 to 100 mg per subject[41]. Significant incidences of thrombocytopenia and major bleed were observed at the high doses of lotrafiban.

Issues in clinical development

Thrombocytopenia with GPIIb/IIIa-receptor inhibitors

Thrombocytopenia has been described with almost all GPIIb/IIIa-receptor inhibitors that have been developed to date[42]. Some of the differences in observed rates of thrombocytopenia could be explained by attributes of the drugs themselves, such as the dose, duration and repetition of exposure, and various drug co-administrations, but might also be predicted by certain baseline patient characteristics. Suggested mechanisms for the thrombocytopenia include immune and non-immune mechanisms.

Bleeding risk

Because of the steep dose–response relationship for all GPIIb/IIIa-receptor antagonists (intravenous and oral), these drugs have a narrow therapeutic window with regard to antiplatelet efficacy or bleeding risk. As they 'thin' the blood, a significant percentage of minor bleeding is evident together with some major bleeding episodes[42].

Monitoring

As already mentioned, standard platelet aggregometry has its problems and limitations and is not a test that has wide-scale applicability. Aggregometry of whole blood and other methods of rapidly assessing platelet function at the point of care have promise in this area[43]. In a clinical

trial, such technology might allow better delineation of the population's pharmacodynamic response to an agent because it could be more widely applied and therefore more patients studied. In clinical practice, patients could be dosed individually so that the range of platelet inhibition believed to provide optimal benefit and safety could be determined.

Interaction between GPIIb/IIIa-receptor antagonists and other anticoagulant/antiplatelet drugs

The interaction between GPIIb/IIIa-receptor inhibitors and anticoagulants (e.g. heparin, hirudin or other antithrombins) and/or other antiplatelet drugs (e.g. aspirin, ticlopidine, clopidogrel) that are usually given concomitantly during and after revascularization procedures might contribute to the clinical efficacy of GPIIb/IIIa-receptor inhibitors. However, this might also lead to enhanced bleeding risks, and hence, careful monitoring and dose adjustment will be essential[42,43].

The future

Future developments might include combined-mode agents, non-peptide thrombin or factor Xa inhibitors with longer lasting action, low-molecular weight heparin, aspirin plus clopidogrel and GPIIb/IIIa-receptor antagonists. There are tremendous challenges ahead with oral platelet GPIIb/IIIa-receptor antagonists in clinical development. Additionally, because aspirin and clopidogrel have proven benefit in reducing vascular events, oral platelet GPIIb/IIIa-receptor antagonists are positioned to provide even greater clinical benefit if tolerated. To optimize efficacy and safety, combination strategies therefore represent great hope.

REFERENCES

1 Ashby, B., Daniel, J.L. and Smith, J.B. (1990) *Platelets Health Dis.* 4, 1–26
2 Davies, M.J. and Thomas, A.C. (1985) *Br. Heart J.* 53, 363–373
3 Fitzgerald, D.J. *et al.* (1986) *New Engl. J. Med.* 315, 983–989
4 Fuster, V., Steele, P.M. and Chesebro, J.H. (1985) *J. Am. Coll. Cardiol.* 5, 175B–184B
5 Hamm, C.W. *et al.* (1987) *J. Am. Coll. Cardiol.* 10, 998–1004
6 Mousa, S.A. and Bennett, J.S. (1996) *Drugs Future* 21, 1141–1154
7 Smitherman, T.C. *et al.* (1981) *Am. J. Cardiol.* 48, 395–402
8 Rubenstein, M.D. *et al.* (1981) *Am. Heart J.* 102, 363–367
9 Theroux, P. *et al.* (1988) *New Engl. J. Med.* 319, 1105–1111
10 Lewis, H.D. *et al.* (1983) *New Engl. J. Med.* 309, 396–403
11 Schwartz, L. *et al.* (1988) *New Engl. J. Med.* 318, 1714–1719
12 Antiplatelet Trialists Collaboration (1994) *Br. Med. J.* 308, 81–106
13 CAPRIE Steering Committee (1996) *Lancet* 348, 1329–1339
14 CAST (Chinese Acute Stroke Trial) Collaborative group (1997) *Lancet* 349, 1641–1649
15 Mousa, S. and Reilly, T. (1995) in *Cardiovascular Disease: Cellular and Molecular Mechanisms, Prevention, Treatment* (Gallo, L.L., ed.), pp. 255–267, Plenum Press
16 Mousa, S. *et al.* (1993) *Cardiology* 83, 367–373
17 EPIC Investigators (1994) *New Engl. J. Med.* 330, 956–961
18 Tcheng, J.E. *et al.* (1995) *Circulation* 91, 215–217
19 Peerlinck, K. *et al.* (1993) *Circulation* 88 (4 Pt 1), 1512–1517
20 Théroux, P. *et al.* (1994) *Circulation* 90, I-232
21 Dondana, P. *et al.* (1996) *Thromb. Res.* 82, 163–175
22 Mousa, S. *et al.* (1994) *Life Sci.* 54, 1155–1162
23 Mousa, S. *et al.* (1994) *Circulation* 89, 3–12
24 Pytela, R. *et al.* (1986) *Science* 231, 1559–1562
25 Philips, D.R., Charo, I.F. and Scarborough, R.M. (1991) *Cell* 65, 359–362
26 D'Souza, S.E. *et al.* (1990) *Biol. Chem.* 265, 3440–3446
27 Pedicord, D. *et al.* (1998) *Thromb. Res.* 90, 247–258
28 Bednar, R.A. *et al.* (1998) *J. Pharmacol. Exp. Ther.* 285, 1317–1326
29 Mousa, S. *et al.* (1993) *Cardiology* 83, 374–382
30 Jackson, S. *et al.* (1994) *J. Am. Chem. Soc.* 116, 3220–3230
31 Rote, W. *et al.* (1993) *J. Cardiovasc. Pharmacol.* 23, 203–211
32 Rote, W. *et al.* (1993) *J. Cardiovasc. Pharmacol.* 23, 194–202
33 Mousa, S.A. and Topol, E. (1997) in *Current Review of Interventional Cardiology* (Vol. 13, 3rd edn) (Serruys, P.W. and Holmes, D., eds), pp. 114–129, Current Medicine
34 Tcheng, J.E. *et al.* (1994) *Circulation* 90, 1757–1764
35 Kleiman, N.S. *et al.* (1993) *J. Am. Coll. Cardiol.* 22, 381–389
36 Kottke-Marchant, K. *et al.* (1995) *Circulation* 92 (8 Suppl.), I-488
37 Cannon, C.P. *et al.* (1998) *Circulation* 97, 340–349
38 Newby, L.K. (1999) *Am. Heart J.* 138, 210–218
39 Muller, T.H. *et al.* (1997) *Circulation* 96, 1130–1138
40 Mousa, S.A. and Wityak, J. (1998) *Cardiovasc. Drug Rev.* 16, 48–61
41 Harrington, R.A. *et al.* (1998) *Circulation* 98 (17 Suppl.), I-251
42 Vorchheimer, D.A. and Fuster, V. (1998) *Circulation* 97, 312–314
43 Quinn, M. and Fitzgerald, D.J. (1998) *Am. Heart J.* 135 (5 Pt 2), S113–S118

Student subscriptions

Students may take out a subscription to *Drug Discovery Today* and receive a 50% discount on the Personal subscription rate. To qualify for this discount please use the bound-in card contained within this journal.