

# Platelets and new antiplatelet drugs

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Platelets play an important, life-saving role in hemostasis and blood clotting at sites of vascular injury. However, unwanted platelet activation and arterial thrombus formation are implicated in the onset of myocardial infarction, stroke and other cardiovascular diseases. Different mechanisms, such as vascular damage, the development of mural platelet thrombi as a response to injury and the biochemical effects of intraplatelet substances that are released in response to damage, may be involved. Thus, antiplatelet therapy has become a mainstay of treatment for these conditions and the benefit of antiplatelet drugs is documented across a wide spectrum of clinical conditions. Aspirin has been regarded as the prototype antiplatelet drug and is still the most widely used agent. Aspirin's antiplatelet effect is directly due to irreversible inactivation of arachidonic metabolism and suppression of thromboxane A<sub>2</sub> synthesis. However, platelet activation occurs via several pathways that do not rely on amplification by released thromboxane A<sub>2</sub>. Therefore, a number of other compounds have been developed to complement the beneficial effect of aspirin. Four main classes of antiplatelet agents are currently available for clinical use: aspirin, phosphodiesterase inhibitors, thienopyridines and the platelet glycoprotein  $\alpha_{IIb}\beta_3$  receptor antagonists. This review discusses state-of-the-art antiplatelet therapies and recent advances, using aspirin as the reference standard.

## Platelets, thrombosis & atherothrombotic vascular disease

Arterial thrombosis rarely occurs in the presence of a healthy or undamaged vascular wall of the active involvement of endothelial cells, which provide a thromboresistant surface for flowing blood [1]. Platelet accumulation in the arterial wall is currently well accepted as a rather early and key event, both in initiation and perpetuation of atherosclerotic lesions.

One of the early events following vascular injury is the adhesion of circulating platelets to exposed subendothelium [2]. Both superficial intimal injury, caused by endothelial denudation, and deep injury, caused by plaque rupture, expose subendothelial collagen and von Willebrand factor (vWF) to platelets of the circulating blood [3]. Platelet adhesion is mediated by the interaction of platelet glycoprotein (GP)Ib/IX receptor with the subendothelial vWF under high shear conditions [4] and by the platelet GPIa/IIa receptor binding to collagen under both stasis and flow [5]. This further triggers a network of signaling events inducing the release and local accumulation of soluble platelet agonists (thromboxane [TX]A<sub>2</sub>, serotonin and ADP and thrombin) [6] and further induces activation and aggregation of platelets by activating specific

receptors. Moreover, platelet activation allows interplatelet contact and the formation of platelet aggregates. This process is mediated by the integrin- $\alpha_{IIb}\beta_{IIIa}$  (GPIIb/IIIa) complex in the platelet membrane, which acts as a promiscuous receptor for several ligands such as fibrinogen, vWF and fibronectin [7]. On the surface of the activated platelet, the GPIIb/IIIa receptor undergoes a conformational change [8], allowing platelets to bind fibrinogen and thus crosslink, which is important for the final common pathway of stable platelet aggregation.

## Platelet adhesion

### *Inhibition of GPIb–von Willebrand factor interaction*

The initial step of thrombus formation consists of platelet adhesion to an injured vessel wall. Especially under the high shear-stress conditions observed in stenosed coronary arteries, the interaction between platelet GPIb and vWF plays a crucial role in platelet-mediated thrombus formation [9]. Blocking GPIb [10–13] or vWF [14–16] with antibodies results in the inability of the platelets to attach to the exposed subendothelium and, thus, the inhibition of the GPIb–vWF interaction has focused considerable attention as an attractive target for the

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prevention of thrombus formation in stenosed arteries [13,16]. Moreover, pharmacologic blockade of the GPIIb–vWF interaction may show a lower bleeding risk than GPIIb/IIIa blockade [13,17]. As shown in a baboon model, the combination of a low dose of a GPIIb inhibitor with a low-dose of a GPIIb/IIIa inhibitor has a potent antithrombotic action with minimal effects on the bleeding time [13]. Given the growing awareness of the importance of inflammation in influencing the outcomes of cardiovascular disease [18], GPIIb inhibitors that reduce platelet–leukocyte interactions might have additional therapeutic benefits. To summarize, anti-GPIIb and anti-vWF may be useful compounds in the therapy of thrombosis and cardiovascular disease. However, the clinical use of GPIIb inhibitors could be limited by potential pathogenic effects on megakaryocytes [19,20] and antibody-induced thrombocytopenia [11,21] and, moreover, only rare data on the use of such inhibitors in clinical trials are currently available [22].

*Inhibition of collagen–platelet interactions*  
Not only the interaction between platelet GPIIb and vWF but also the interaction between collagen and vWF plays a crucial role under conditions of high shear rates, which is typically found in the arteriolar circulation and at sites of arterial stenosis [23]. Collagen, the most thrombogenic component of the extracellular matrix, directly binds to receptors that mediate platelet adhesion, and induces

activation and aggregation [24,25]. The binding site for collagen on vWF is localized to the A3 domain and blocking this domain from binding to collagen by a specific antibody reduced thrombus growth *in vivo* and prolonged skin-bleeding time [26]. Thus, collagen antagonists could be potent antiplatelet agents, targeting platelet adhesion by inhibiting the collagen–platelet interaction. However, as reviewed recently, these selective antagonists might have potential limitations as they could have limited antithrombotic protection in patients with arterial thrombosis [27]. Indeed, it has been shown that most patients with acute myocardial infarction (MI) have plaque rupture and non-occlusive thrombus formation up to a week before the clinical event [28] and, thus, collagen antagonists might be restricted to specific subsets of patients with vascular disease.

**Platelet aggregation**

*Aspirin & related cyclooxygenase inhibitors or thromboxane antagonists*

*Aspirin*

Aspirin has been regarded as the prototype antiplatelet drug, with multiple dose-dependent therapeutic effects, and is still the most widely used and studied agent. Aspirin irreversibly inhibits arachidonate cyclooxygenase (COX) activity in platelets, thereby reducing the extent of TXA<sub>2</sub> formation that occurs after activation of phospholipase A<sub>2</sub> and release of arachidonic acid (AA) (Figure 1) [29].

However, although aspirin effectively reduces platelet secretion and aggregation, it is a relatively weak platelet inhibitor [30]. Other platelet-dependent prothrombotic mechanisms are less affected or not modified at all at doses that block platelet-dependent TX formation. For example, aspirin does not inhibit shear stress-induced platelet activation and adhesion [31], does not inhibit α-granule secretion in response to ADP and other agonists, and does not inhibit fibrinogen binding [32,33]. Moreover, aspirin usage is associated with potentially life-threatening side effects such as gastric hemorrhage [34]. Thus, researchers have been encouraged to investigate and develop new antiplatelet drugs that are equivalent in strength to aspirin, but with fewer, or no, adverse events (Table 1).

*Acute therapy & secondary prevention of cardiovascular disease & stroke*

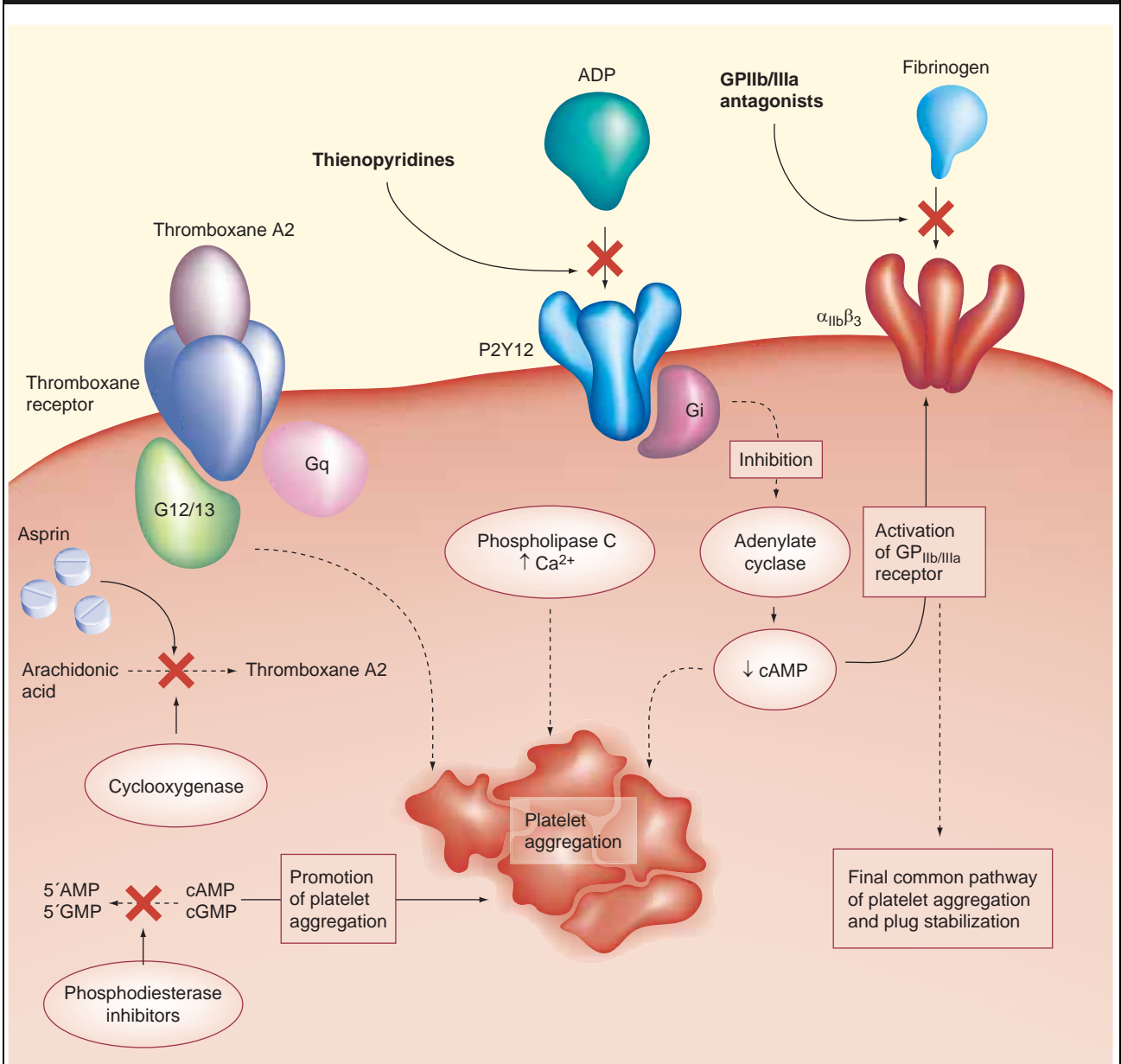
Since the 1950s when it was recognized that aspirin could reduce the incidence of MI [35], multiple, randomized, controlled clinical trials have shown a clinically significant decrease in cardiovascular

**Table 1. Antiplatelet agents.**

Pathways	Agents
<b>Platelet adhesion</b>	
von Willebrand factor	GPIIb-receptor antibody, von Willebrand factor antibody
<b>Platelet aggregation</b>	
Cyclooxygenase pathway	
• Cyclooxygenase	Aspirin, NO-aspirin, gingerols
• Thromboxane A2 synthase	Dazoxiban
• Thromboxane A2 receptor	Vapiprost
• Thromboxane synthase and receptor	Ridogrel, terbogrel, picotamide
• Prostacyclin	Beraprost, iloprost, ebaprostanol
Phosphodiesterase	Dipyridamole and cilostazol
ADP	P2Y12 receptor antagonists and P2Y1 receptor antagonists
GPIIb/IIIa	Murine– human antibodies, synthetic peptide forms and synthetic nonpeptide forms

GP: Glycoprotein; NO: Nitric oxide.

Figure 1. Platelet receptors and their antagonists.



Thromboxane A<sub>2</sub> is produced from arachidonic acid via cyclooxygenase and is released from the platelet to work with the thromboxane receptor. The thromboxane receptor (a G-protein coupled receptor) causes platelet aggregation via both Gq and G12/13. Aspirin irreversibly inhibits cyclooxygenase, for the lifetime of the platelets, preventing thromboxane production. Phosphodiesterase inhibitors prevent the breakdown of cAMP and cGMP. Therefore cAMP and cGMP levels are maintained inhibiting platelet aggregation. The ADP P2Y<sub>12</sub> receptor (a G-protein coupled receptor) is linked to adenylate cyclase via Gi. Upon stimulation the adenylate cyclase is inhibited, leading to a fall in cAMP and promotion of platelet aggregation. Thienopyridines irreversibly bind to the ADP P2Y<sub>12</sub> receptor preventing the inhibition of adenylate cyclase, therefore maintaining cAMP levels and inhibiting platelet aggregation. The GPIIb/IIIa antagonists prevent fibrinogen binding to the  $\alpha_{IIb}\beta_3$  receptors and therefore inhibit the 'final common pathway of platelet aggregation'. cAMP: Cyclic adenosine monophosphate; cGMP: Cyclic guanosine monophosphate.

morbidity and mortality in patients at risk of recurrent atherothrombotic events [36–39].

The most accurate data regarding the efficacy of aspirin comes from the Antiplatelet Trialists' Collaboration (ATC), a meta-analysis of 287

randomized studies of various antiplatelet regimes, which demonstrated that aspirin caused a 25% reduction in cardiovascular outcomes (non-fatal MI, strokes or vascular death) in patients with pre-existing cardiovascular disease [39]. The

Second International Study of Infarct Survival (ISIS-2) has established the benefit in acute MI [36,37]. Patients (n = 17187) with acute MI were randomized to one of four arms of therapy consisting of placebo, aspirin, streptokinase or streptokinase plus aspirin. At the end of 5 weeks, patients receiving aspirin therapy alone had a significant (23%) reduction in vascular mortality and a nearly 50% reduction in the risk of nonfatal reinfarction and nonfatal stroke. Administration of streptokinase alone was associated with a 25% reduction in vascular deaths, and the combination of streptokinase and aspirin was significantly better than either agent alone (42% reduction in vascular mortality with combination therapy). Interestingly, the mortality benefit of combined aspirin and streptokinase therapy was maintained after 10 years' follow-up [37].

In contrast to the secondary prevention of MI, the therapeutic value of aspirin in preventing ischemic stroke is less clear and appears to be related to the cause and severity of cerebral ischemia [40–42]. Two large, randomized trials of aspirin use in the setting of acute, ischemic stroke have demonstrated that the use of aspirin reduces both recurrent stroke and the combined incidence of death or nonfatal stroke [43,44](Table 2). The relative risk reduction (RRR) in fatal or nonfatal vascular events was only 10% in this setting. Accordingly, a meta-analysis showed a modest 13% RRR of vascular events in patients with cerebral ischemia of arterial origin [45]. Overall, the benefit of aspirin in acute stroke treatment and secondary prevention of stroke are definite but modest.

*Primary prevention of cardiovascular disease & stroke*

Although the beneficial effect of aspirin in the secondary prevention of ischemic events is well established [39], the role of primary prevention is

less clear. A meta-analysis of five randomized controlled trials of aspirin for primary prevention demonstrated that aspirin was associated with a statistically significant, 32%, reduction in the risk of a first MI and a significant, 15%, reduction in the risk of all important vascular events, but had no significant effects on nonfatal stroke or vascular death [46,47]. Interestingly, the absolute benefit of aspirin clearly increases with the risk of cardiovascular events in the treatment group [48]. Additionally, aspirin increases the risk for hemorrhagic strokes and major gastrointestinal bleeding [46]. Data regarding the use of aspirin for the primary prevention of strokes in high-risk patients are not encouraging [49,50]. Overall, low-dose aspirin appears to decrease the risk of MI in men with little effect on the risk of stroke.

Since three of the previous five primary prevention trials [49–53] exclusively evaluated men, and fewer than 180 of the 2042 vascular events occurred in women, the currently published results of the Women's Health Study are of interest [54]. Indeed, the current recommendations for the use of aspirin in primary prevention in women are based on limited direct data from women [46,55,56]

In the Women's Health Study [54] there was a nonsignificant RRR of 9% in the primary outcome of first major cardiovascular events (i.e., MI, nonfatal stroke or death from a cardiovascular event). Regarding the individual end points, women in the aspirin group had a 17% RRR (p = 0.04) in stroke, compared with the placebo group, owing to a 24% RRR of ischemic stroke (p = 0.009) and a nonsignificant increase in the risk of hemorrhagic stroke. Moreover, aspirin therapy was associated with a 22% RRR of transient ischemic attack (TIA) (p = 0.01) and a significant 19% RRR of nonfatal stroke (p = 0.02). However, compared with placebo, aspirin had no significant effect on the risk of

**Table 2. Trials of aspirin therapy in acute ischemic stroke [48].**

End point	Chinese acute stroke trial [43]			International stroke trial [44]		
	Aspirin (%)	No aspirin (%)	2P	Aspirin (%)	No aspirin (%)	2P
Death	3.3	3.9	0.04	9.0	9.4	NS
Death and nonfatal CVA	5.3	5.9	0.03	11.3	12.4	<0.05
Recurrent CVA	1.6	2.1	0.01	2.8	3.9	<0.001
Hemorrhagic CVA	1.1	0.9	NS	0.9	0.8	NS

Values are given as percentages with the exception of 2P values. CVA: Cerebrovascular accident. NS: Not significant.

fatal stroke, fatal or nonfatal MI or death from cardiovascular causes. In a subgroup analysis, the most consistent benefit of aspirin was observed among the subgroup of women 65 years of age or older at study entry. This subgroup showed a 26% RRR ( $p = 0.008$ ) in major cardiovascular events, a 30% RRR ( $p = 0.05$ ) in ischemic stroke, and a 34% RRR ( $p = 0.04$ ) in MI. As expected, the rates of any gastrointestinal (GI) bleeding were higher in the aspirin group than in the placebo group (4.6 vs. 3.8%,  $p < 0.001$ ) but without significantly more episodes of fatal GI bleeding.

In summary, questions remain concerning the optimal therapeutic dose of aspirin, and concerning its use in the primary prevention of vascular disease. Moreover, the reasons for any sex-based differences in the efficacy of aspirin for primary prevention are unclear and require further explorations. In patients with a relatively low risk of developing cardiovascular (or cerebrovascular disease), the risk of prophylactic aspirin therapy may be outweighed by the risk of hemorrhagic complication. Conversely, in high-risk patients, the benefits of therapy may outweigh the risks of hemorrhagic complications. Indeed, recent studies have suggested that the indications for aspirin use should be expanded to primary prevention in populations at high risk, including diabetes, carotid stenosis, peripheral vascular disease, end-stage renal disease [47,57,58] or polycythemia vera [59]. Notwithstanding these results, it remains essential to balance the cardio/cerebrovascular risk profile against the risk of potential bleeding complications for each patient (irrespective of gender) when prescribing aspirin [46,54,60–62].

#### *Recommendations for aspirin use*

Overall, aspirin remains the background template therapy, both for acute ischemic syndromes and secondary prevention after MI, stroke or TIA and

in patients with chronic stable angina (Table 3). Low-dose aspirin (75–150 mg) is an effective antiplatelet regimen for long-term prevention of serious vascular events, whereas in clinical situations, where an immediate antithrombotic effect is required (such as acute MI, stroke or unstable angina pectoris), a loading dose of at least 150 to 325 mg is recommended [39,48,60,63–65]. For primary prevention, no clear indications currently exist, although low-dose aspirin is recommended, especially in those patients believed to be at high risk for the development of cardiovascular disease and stroke [48,55,61,65].

In conclusion, individual trial data shows substantial heterogeneity in the treatment of cardiovascular/cerebrovascular diseases but the reason for these differences in aspirin therapy is still unknown. However, increased platelet-dependent TX generation may only occur in a minority (30%) of patients with acute ischemic stroke [66,67] and a variable importance of TXA<sub>2</sub> as a mechanism to amplify the hemostatic response to plaque destabilization in different clinical settings has been suggested [62]. Thus, one may speculate that the TX-mediated amplification of platelet response to acute vascular injury plays a more important role in cardiovascular than in cerebrovascular diseases, which might therefore explain the superior benefit of aspirin in the treatment of cardiovascular diseases.

#### *Optimal dose of aspirin*

The overall results of clinical trials in which different doses of aspirin have been tested indicate that lower doses of aspirin decrease the prevalence of GI side effects [62]. A direct comparison on the prevalence of GI toxicity among patients using 300 and 1200 mg aspirin once daily in the UK-TIA trial [68] showed that both subjective GI complaints and GI bleeding were more frequent at 1200 mg/day than at 300 mg/day. In the 30-mg group of the Dutch TIA [69] trial, major bleeding complications (requiring hospital attendance) were slightly less common than in the 300-mg group (RRR 23%) and significantly fewer minor bleeds occurred (RRR 42%). Gastric discomfort or unspecified side effects were reported significantly less often with 30 mg than with 300 mg of aspirin. Another study comparing clopidogrel with placebo on top of a 'background' of aspirin therapy (aspirin doses ranging from 75 to 352 mg/day were used in the Clopidogrel in Unstable angina to prevent Recurrent ischemic Events [CURE] trial), demonstrated that bleeding risk increased with increasing aspirin dose, with or without clopidogrel [70].

**Table 3. Summary of recommended uses for aspirin.**

Clinical indication	Recommended dose
Acute MI, stroke, or unstable angina pectoris	Loading dose of at least 150 mg (up to 325 mg)
Secondary prevention after MI, nonhemorrhagic stroke, or TIA and in patients with chronic stable angina	Daily therapy with 75–150 mg
Primary prevention	No clear indication at this time. Consider therapy with 75–160 mg/day in patients believed to be at high risk for the development of cardiovascular disease.

MI: Myocardial infarction; TIA: Transient ischemic attack.

In contrast, the accumulated evidence from clinical trials makes it clear that aspirin doses from 75 to 1300 mg do not (generally) alter the clinical benefit [39] (Table 4). For example, The Acetylsalicylic Acid and Carotid Endarterectomy trial [71] reported that the risk of the composite outcome of MI, stroke, or death within three months of carotid endarterectomy was significantly lower among patients taking 81 or 325 mg aspirin daily than in those taking 625 to 1300 mg. CURE investigators showed that the bleeding risk increased with increasing doses of aspirin, with or without clopidogrel, but without any increase in efficacy [70]. Moreover, aspirin doses of less than 75 mg/day have been less widely assessed than those of 75 to 1500 mg/day and seemed to have a somewhat smaller effect at doses lower than 75 mg/day [39] (Table 4).

Summarizing, any effective antiplatelet dose of aspirin is associated with an increased risk of bleeding and thus, the individual benefit:risk ratio determines the dose of the compound and use of the lowest effective dose of aspirin (Table 3) is probably the most rational strategy to maximize efficacy and minimize toxicity.

#### *Negative effects of aspirin on angiotensin-converting enzyme inhibitors*

Aspirin and angiotensin-converting enzyme (ACE) inhibitors are widely used in combination to treat a wide spectrum of cardiac disorders. There is experimental evidence showing that ACE inhibitors limit the development of infarct size, reduce the incidence of ischemic and reperfusion arrhythmias, and enhance the recovery of contractile function of stunned myocardium [72]. The cardioprotective effects of ACE inhibitors are mediated by an attenuated degradation of bradykinin [73,74]. Bradykinin, a potent vasodilator on its own, activates vascular endothelial B<sub>2</sub>-kinin receptors, which promote the formation of vasodilatory prostaglandins (PGs) through the action of phospholipase-A<sub>2</sub> and COX [75,76]. Thus, drugs that

inhibit endothelial COX, such as aspirin, may reduce the synthesis of vasodilatory PGs. Accordingly, the inhibition of COX may reduce the efficacy of ACE inhibition and therefore the safety of combination therapy with aspirin and ACE inhibitors has been questioned because both drugs affect a related PG-mediated pathway.

However, the results of several trials are controversial concerning the negative interaction of the combined therapy of ACE inhibitors with aspirin. *Post hoc* analysis of two large, multicenter trials have suggested that aspirin causes blunting, or even complete abrogation, of the benefit of ACE inhibitors on mortality in patients with heart failure [77,78]. In another retrospective analysis, ACE inhibitors were associated with an increased mortality in patients who took aspirin following percutaneous coronary intervention (PCI) [79]. More recently, no evidence of a negative therapeutic interaction between aspirin and ACE inhibitors was found in stable patients with chronic heart failure related to left-ventricular systolic dysfunction [80]. Similar interactions have not been found in patients with MI [81,82] and the negative hemodynamic effects may be seen with high doses of aspirin only. Indeed, several studies have found that a negative interaction with ACE inhibitors is present with high doses of aspirin (i.e., 325 mg) [79,83–85], but not with low doses (i.e., 100 mg) [80,86,87]. Although the controversy has not yet been resolved, the recommendation of low-dose aspirin in patients with chronic heart failure seems to be justified [80,88].

#### *Nitric oxide-releasing aspirin*

As aspirin can cause severe damage to the stomach, a nitric oxide (NO)-releasing derivative (NCX-4016) has been developed that might have clinical promise in the protection from atherosclerosis without the unwanted effects on the stomach. NO protects the gastric mucosa, induces vasodilatation, and inhibits platelet

**Table 4. Aspirin doses reducing vascular events in high-risk patients\*.**

Dose of aspirin (mg/day)	Number of trials	Number of patients	Odds reduction <sup>†</sup> (%)
<75	3	3.655	13 ± 8
75–150	12	6.776	32 ± 6
160–325	19	26.513	26 ± 3
500–1500	34	22.451	19 ± 3

\*Data from Antithrombotic Trialists Collaboration [39].

<sup>†</sup>Data are presented as mean ± standard deviation.

aggregation, inflammation, cellular proliferation and apoptosis through both a cyclic guanosine monophosphate (cGMP)-dependent and -independent mechanism [89]. In experimental animal studies, NO aspirin had antiatherosclerotic and antioxidant effects in the arterial wall of hypercholesterol mice and inhibited restenosis after PCI in rats [90,91]. In another rat model, NO-releasing aspirin reduced brain damage after focal cerebral ischemia, indicating that NO release associated with aspirin confers neuroprotective effects against ischemic injury [92].

Recently, a randomized, parallel-group, clinical trial was designed to assess whether NO-releasing aspirin could have broader anti-inflammatory and antithrombotic effects, as well as better gastric tolerability than aspirin [93]. A total of 48 healthy subjects were randomized to receive NO-releasing aspirin 800 mg twice daily, NO-releasing aspirin mg twice daily plus aspirin 325 mg, aspirin 325 mg, or placebo for 21 days. In this study, NO-releasing aspirin was equally effective as aspirin in inhibiting COX activity but caused less gastric damage. These beneficial effects of NO-releasing aspirin, combined with the inhibition of TXA<sub>2</sub>, are very interesting. As atherogenesis and plaque rupture are points on the continuum of vascular inflammation, one may speculate that the NO aspirin, NCX-4016, could be superior to aspirin in the primary and secondary prevention of vascular events and in gastric tolerability. Furthermore, NO-releasing aspirin may offer an attractive alternative in patients in whom COX inhibition with aspirin may be poorly tolerated. Thus, larger multicenter clinical trials are warranted in order to establish the clinical efficacy and safety of the NO-releasing aspirin, NCX-4016.

*Nonaspirin, nonsteroidal anti-inflammatory drugs in cardiovascular disease*

Although a variety of traditional nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit TXA<sub>2</sub>-dependent platelet function through competitive, reversible inhibition of COX-1 the effects of these NSAIDs on cardiovascular disease are controversial. An excellent review has recently been published that addresses this problem [62]. For instance, naproxen was shown to have a protective effect against acute MI [94] and to reduce the risk of thromboembolic cardiovascular events among patients with rheumatoid arthritis [95]. However, other population-based studies [96–98] have failed to detect protective effects of naproxen or other nonaspirin NSAIDs, considering that none of these

drugs should be used for cardioprotection in the absence of evidence from randomized controlled trials to lend support to such a practice. The only nonaspirin NSAIDs that have been tested for their antithrombotic efficacy in randomized trials are sulfinpyrazone, indobufen, flurbiprofen and triflusal; however, none of these drugs have been approved as an antiplatelet drug since the overall results of these trials were not satisfactory [39,62].

Another interesting point of nonaspirin NSAIDs is the fact that they may interact with the cardioprotective effects of aspirin. Based on epidemiologic data, some people have recommended avoiding ibuprofen in patients taking aspirin and suggested that ibuprofen reverses the cardioprotection offered by aspirin [99]. Indeed, results from an *in vivo* study demonstrated an interaction between aspirin and ibuprofen on platelet function, but no such interaction was reported with rofecoxib, paracetamol or diclofenac [100]. The hypothesis that ibuprofen may interact with the cardioprotective effects of aspirin, at least in patients with established cardiovascular disease, is a theory that has been supported by the findings of a recently published trial [101]. This study involved 7107 patients who received aspirin alone, aspirin and ibuprofen, aspirin plus diclofenac or aspirin plus other NSAIDs. Patients in the aspirin plus ibuprofen group had a significantly higher risk of all-cause mortality ( $p = 0.0011$ ) and cardiovascular mortality ( $p = 0.0305$ ) than those in the aspirin-alone group. No such increased risk was noted in users of aspirin plus diclofenac or aspirin plus other NSAIDs.

However, as reviewed very recently [102] no randomized, controlled trials addressing this particular issue exist at the moment and the data obtained from observational and epidemiologic studies are conflicting and limited.

*Cyclooxygenase-2 inhibitors in cardiovascular disease*

Selective COX-2 inhibitors (coxibs) differ from traditional NSAIDs in two major ways. Coxibs are less likely to result in NSAID-induced gastropathy, and they do not inhibit platelet function [103,104]. The major benefits of coxibs are the reduction in gastric ulcer formation and bleeding from those ulcers, as reported by the VIOxx Gastrointestinal Outcomes Research (VIGOR) trial [105]. Another benefit of the platelet-sparing coxibs is their use as analgesics and anti-inflammatory agents in situations in which bleeding may limit the use of traditional NSAIDs, such as trauma and surgical procedures [106,107]. Otherwise, coxibs are

supposed to have several effects that could increase the risk of cardiovascular disease, including a decrease in prostacyclin levels, increasing blood pressure, decreasing angiogenesis and destabilizing plaque [108].

Recently, this class of drugs has come under scrutiny due to clinical reports of an associated increased risk of serious cardiovascular events [109,110]. As reported in the VIGOR trial, there were more cardiovascular events among patients given a high dose of rofecoxib than among those patients given naproxen, a nonselective NSAID with platelet-inhibiting properties of unclear clinical relevance [105]. In contrast, pooled data from other randomized trials have not shown a significant difference in cardiovascular risk between rofecoxib and placebo or other nonselective NSAIDs [111–113]. Indeed, most of the earlier trials of coxibs did not appear to show an increase in cardiovascular events [114–116]; however, these trials were generally short-term studies designed to assess the use of this class of drug for pain relief and to evaluate associated adverse GI events.

Overall, studies have provided conflicting data on the association of coxibs with cardiovascular risk but only limited long-term data have been available for analysis so far. However, this observation has been changed since the results of three long-term trials have recently been published [108,117,118]. The Adenomatous Polyp Prevention On Vioxx (APPROVe) study, which was designed to determine the effect of 3 years' treatment with rofecoxib on the risk of recurrent neoplastic polyps among patients with a history of colorectal adenomas, showed an increased cardiovascular risk associated with the long-term use of rofecoxib [108]. The Coronary Artery Bypass Grafting (CABG) surgery study showed that cardiovascular events (including MI, cardiac arrest, stroke and pulmonary embolism) were more frequent among the patients given parecoxib and valdecoxib than among those given placebo (2 vs. 5%; risk ratio: 3.7;  $p = 0.03$ ) [118]. The Adenoma Prevention with Celecoxib (APC) study reviewed all potentially serious cardiovascular events among 2035 patients with a history of colorectal neoplasia who were enrolled in a trial comparing two doses of celecoxib (200 and 400 mg twice daily) with placebo for the prevention of colorectal adenomas [117]. There was a dose-related increase in cardiovascular events for celecoxib when compared with placebo. Of interest, the results of a randomized, controlled clinical trial of celecoxib with Alzheimer's disease, reported to the US Food and

Drug Administration (FDA), demonstrated an increase in cardiovascular events among patients receiving celecoxib [401].

Summarizing, since different coxibs were found to be associated with cardiovascular complications, it appears that this is a class effect. The risk of serious cardiovascular events will need to be weighed against any potential benefits of coxibs in preventing colorectal neoplasia and in relieving pain. The lesson to be learnt from this observation has been discussed very recently [119].

#### *Aspirin-related drugs*

Given the presumed understanding of aspirin's mode of antiplatelet effect, it should be possible to design a more specific drug to interfere with the COX/TX pathway. Aspirin, by nonselectively blocking COX both in platelets and in endothelial cells, not only inhibits the TXA<sub>2</sub> pathway of platelet activation but, at the same time, also the generation of vasodilating and platelet-inhibitory prostanoids, such as PGI<sub>2</sub> (or prostacyclin), by the endothelial cells. This is of importance because there is an increased intravascular prostacyclin generation in patients at advanced stages of atherosclerosis, which is paralleled by the degree of platelet activation [120]. Even 40-mg doses of aspirin are sufficient to inhibit the local production of antithrombotic PG generation in the blood vessels of atherosclerotic patients [121]. This appears to be a limitation to the antithrombotic efficacy of aspirin and was one of the reasons to look for alternatives, specifically, more selective inhibitors of TX formation and action.

#### *Agents interfering directly with thromboxane formation &/or action*

TX synthase inhibitors and TX receptor antagonists directly interfere with TX formation (synthase inhibitors) and action (receptor blockers). TX synthase inhibitors do not reduce vascular PGI<sub>2</sub> formation but enhance it by shifting accumulating PG endoperoxidase into this pathway [122]. In fact, TX synthase inhibitors and TX receptor blockers have been demonstrated to exhibit potent antithrombotic effects in several animal studies [123–129]. In general, inhibition of TX synthase appears to be more efficient than the blockade of TX receptors [126,130]. To summarize, all of these data suggest that the selective inhibition of TX synthase, in particular if combined with the blockade of TX receptors, would be a useful approach to antiplatelet therapy.

Picotamide, a dual TXA<sub>2</sub> synthase inhibitor/receptor antagonist, slowed the evolution of



early carotid lesions in a controlled study in diabetic patients [131]. However, more well-proven randomized controlled trials are required to further investigate the possible benefit of picotamide in preventing the formation of arterial thrombus in such patients.

The Coronary Artery Restenosis Prevention On Repeated Thromboxane-antagonism (CARPORT) study compared the TX receptor antagonist vapiprost with aspirin in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). After a 6-month follow-up period, no effect on the incidence of restenosis was observed [132].

The combined TX synthase inhibitor and TX receptor antagonist, ridogrel, was compared with aspirin in patients with acute MI receiving streptokinase in the Ridogrel versus Aspirin Patient Trial (RAPT) [133]. Ridogrel was not superior to aspirin in enhancing the fibrinolytic efficacy of streptokinase, although there was a lower rate of new ischemic events noted in a *post hoc* analysis.

Moreover, terbogrel, another combined TX synthase inhibitor/receptor antagonist failed to live up to expectations in a trial of patients with primary pulmonary hypertension [134]. Although terbogrel was able to reduce TX metabolism by 98% with a modest, but statistically insignificant (39%) rise in PGI<sub>2</sub> metabolites, it was associated with severe leg pain, thus limiting its clinical utility. In contrast, another study of TX synthase inhibition in patients with primary pulmonary disease did not describe leg pain as a side effect [135] and, in a recently published trial, terbogrel was shown to be well tolerated without obvious adverse effects in healthy volunteers [136].

In summary, despite showing considerable promise in preclinical studies, TX synthase inhibitors and TX receptor antagonists have been disappointing in clinical trials and have not demonstrated a benefit over aspirin. Therefore, these newer drugs could provide an alternative approach to antiplatelet therapy, but more clinical trials are required.

#### *Prostacyclin-related agents*

PGI<sub>2</sub>, known to have potent antiplatelet and vasodilatory activities [137], occurs naturally in the vascular endothelium and has been approved by the FDA for the management of pulmonary artery hypertension [138–140]. PGI<sub>2</sub> relaxes vascular smooth muscle [141,142], inhibits platelet aggregation [141,142] and also suppresses vascular

smooth muscle proliferation [143]. Thus, one may speculate that this class of drugs could also have therapeutic potential in the treatment of cardiovascular or cerebrovascular diseases.

The PGI<sub>2</sub> analog iloprost was, however, unable to prevent reocclusions of stenosed dog coronary arteries after electrical injury or thrombolysis [144]. This could be due to receptor desensitization owing to high levels of PGI<sub>2</sub>. Importantly, platelet receptors may become downregulated if there is a significant increase in PGI<sub>2</sub> production, for example, in unstable angina and acute MI [145,146]. Moreover, only a small number of larger clinical trials with PGI<sub>2</sub>-related compounds [147,148] exist and the results, in general, were not encouraging. In a more recent study the orally active PGI<sub>2</sub> analog, beraprost, was compared with placebo in patients with intermittent claudication [149]. Although the incidence of critical cardiovascular events was not significantly reduced in those patients assigned to beraprost, there was a significant reduction in the combination of cardiovascular death and MI. Critical cardiovascular events were defined as:

- Death of cardiovascular origin, nonfatal MI or unstable angina
- Stroke or TIA
- Critical leg ischemia, subacute critical ischemia, peripheral angioplasty, peripheral bypass surgery or amputation at any level

Nonetheless, judgements on whether PGI<sub>2</sub> analogs could have potential beneficial cardioprotective effects should not be made from this one trial. Moreover, major problems include the receptor-mediated nature of response and the low selectivity for the platelet, which may result in nonspecific effects, for example hypotension [122].

To summarize, the future of these drugs as a possible potential new approach to the treatment of vascular diseases is, indeed, questionable.

#### *Gingerols & related analogs*

Gingerols, the active components of ginger (the rhizome of *Zingiber officinale*, Roscoe), were shown to selectively inhibit secondary platelet activation and ATP release from platelets in human platelet-rich plasma, which is due to inhibition of AA metabolism and COX activity [150]. Gingerols and other synthetic analogs were also shown to have a strong COX-1 inhibitory activity in rat basophilic leukemia cells [151], a cell line with COX-1 expression [152]. More recently, the inhibition of AA-induced

platelet activation in human blood was studied. Gingerol and gingerol analogs dose-dependently inhibited COX-1 activity and the COX-1 inhibitory effect of these substances was more potent than aspirin [153]. In a rat model, ginger extract and other ginger preparations showed antiulcer activity [154,155]. Thus, these substances could be useful well-tolerated platelet activation inhibitors; however, data on these substances are rare and additional experiments are needed to gain a better insight into the effect of platelet inhibition by gingerols.

#### *Phosphodiesterase inhibitors*

##### *Dipyridamole*

Both the inhibition of cyclic nucleotide phosphodiesterase (PDE) (the enzyme that degrades cyclic adenosine monophosphate [cAMP] to 5'-AMP, resulting in the intraplatelet accumulation of cAMP, a platelet inhibitor) and the blockade of the uptake of adenosine (which acts at A<sub>2</sub> receptors for adenosine to stimulate platelet adenylyl cyclase and, thus, increases the level of cAMP), have been suggested [156]. Moreover, dipyridamole blocks the enzyme cGMP PDE, thereby inhibiting the breakdown of cGMP (Figure 1)[157]. Raised levels of cAMP and cGMP within platelets potentiate inherent mechanisms, resulting in vasodilatation and inhibition of aggregation [158].

##### *Dipyridamole in cerebrovascular disease*

Dipyridamole appears to have similar efficacy to low-dose aspirin in preventing stroke [159]. In patients with cerebrovascular disease, the European Stroke Prevention Study (ESPS)-2 demonstrated that the combination of low-dose aspirin (50 mg daily) and extended-release dipyridamole (ERDP) (400 mg daily) was superior in preventing nonfatal stroke than either drug alone [159]. However, comparing dipyridamole plus aspirin with aspirin alone was associated with only a nonsignificant reduction in serious vascular events [39,160]. Indeed, the apparent reduction in nonfatal stroke was derived mainly from the ESPS-2 study but this result was not supported by the findings for nonfatal stroke in other studies or by the overall findings for nonfatal MI or vascular death [39]. Finally, headaches limited the use of aspirin/ERDP as they occurred in 37% of treated patients in the ESPS-2 study and resulted in a high rate of noncompliance.

Currently, aspirin/ERDP is being compared with clopidogrel in the largest ever secondary stroke-prevention trial: the Prevention Regimen

For Effectively avoiding Second Strokes trial (PROFESS) [161]. Irrespective of the outcome of this study, there is one major limitation in the design of the PROFESS study, as the two treatment arms will not be compared against aspirin. This aspect is important since the evidence of the superiority of aspirin/ERDP over aspirin alone hinges on a single trial. Moreover, in high-risk patients with recent TIA or ischemic stroke, clopidogrel plus aspirin was not superior to clopidogrel alone, as demonstrated in the Management of ATherothrombosis with Clopidogrel in High-risk patients with recent TIA or ischemic stroke (MATCH) study, but was not compared with aspirin alone. Thus, the results of the PROFESS trial will not enable a judgement to be made on the benefits of a combination of different antiplatelet drugs as a therapeutic approach for patients with cerebrovascular diseases.

In conclusion, aspirin/ERDP has been FDA approved and is usually classified as a potential first-line therapy in the secondary prevention of ischemic stroke and TIA, especially in patients with lower cardiovascular comorbidity [162,163] but the current feeling is that there is not yet sufficient evidence to justify adoption of aspirin and dipyridamole as a first-line treatment for the secondary prevention of stroke. Thus, aspirin should be the first-line antiplatelet therapy in the secondary prevention of stroke and TIA [39,164,165] (Table 3) until prospective, randomized clinical trials have shown a sustained benefit of aspirin/ERDP or clopidogrel plus aspirin over the gold-standard treatment with aspirin.

##### *Dipyridamole & coronary artery steal*

Perfusion imaging during coronary vasodilatation with either adenosine or dipyridamole is widely used for the diagnosis of coronary artery disease (CAD) [166–168]. Dipyridamole, administered intravenously, represents a well-established medication that induces dilatation of coronary arteries by inhibiting the degradation of adenosine.

Vasodilatation in nonischemic regions can divert blood from already underperfused regions to parallel-perfused regions where the vasodilator reserve has not been exhausted (coronary steal) [169]. Coronary steal has been well described in canine studies of CAD after intravenous dipyridamole [170]. Moreover, myocardial ischemia due to coronary steal is generally believed to be manifested clinically by ST segment depression following coronary vasodilatation [171,172].

Indeed, there are some reports concerning patients who developed angina pectoris manifested by ischemic electrocardiographic changes and perfusion defects, which occurred after dipyridamole administration [173–175]. Moreover, a significant difference on coronary angiography between patients with dipyridamole-induced angina pectoris and those without angina pectoris was found in the presence of collaterals ( $p < 0.05$ ) [173]. Therefore, it has been suggested that angina pectoris during dipyridamole stress test is due to ischemia, which is not related to the severity of CAD but is probably owing to coronary steal to the collateralized territory in patients without transmural MI. Thus, dipyridamole-induced angina pectoris could be predictive for collaterals and may indicate viability in patients with MI [173].

Overall, since dipyridamole has a vasodilatory effect, it should be used with caution in patients with severe CAD (e.g., unstable angina and MI) and chest pain may be aggravated in patients underlying CAD who are receiving dipyridamole.

#### *Cilostazol*

Cilostazol, a potent inhibitor of platelet aggregation [176] with vasodilating properties, inhibits cAMP-selective PDE-III [177], thereby increasing the intracellular level of cAMP. Cilostazol reversibly inhibits platelet aggregation induced by a variety of stimuli, including thrombin, ADP, collagen, AA, epinephrine and shear stress [178]. Cilostazol has also been shown to be a potent antiplatelet agent with antiproliferative properties [179], that increases peripheral blood flow [180] and ameliorates insulin resistance [181,182]. Thus, it has been suggested that cilostazol could prevent both thrombosis and restenosis after coronary stenting when coadministered with aspirin [183,184] but it could also be effective in the primary prevention of ischemic stroke in subjects with Type II diabetes mellitus [185].

Several reports have demonstrated that cilostazol can prevent subacute thrombosis after stent implantation and may reduce restenosis after coronary interventions [186–189]. As reported [190], after coronary angioplasty, the restenosis rate was significantly lower in groups with cilostazol than in groups with aspirin or ticlopidine. Moreover, cilostazol has been shown to have an outstanding effect on the prevention of acute or subacute thrombotic complication after coronary stenting, equal to ticlopidine [191]. A very recent study demonstrated that ticlopidine showed significantly less subacute thrombosis after stenting compared with cilostazol but the inhibition of neointimal

proliferation was greater in the cilostazol than in the ticlopidine group [192]. Moreover, a meta-analysis of five clinical studies comparing cilostazol (plus aspirin) with ticlopidine (plus aspirin) showed no difference regarding the effectiveness and safety for a 1-month period when used as an adjunctive therapy after coronary stenting [193].

Despite the obvious beneficial effect of cilostazol in these clinical settings, there may be concerns regarding the study designs of these trials. Indeed, these trials were single-center studies with a relatively small number of patients [186–192] and some of them were not randomized [190] or double blinded [188,192]. Thus, large-scale, multi-center, randomized trials are needed to confirm the efficacy of cilostazol in other patients groups of different ethnicity.

In animal studies, cilostazol was shown to decrease ischemic brain infarction [194–196]. In a recently published study of guinea pig and human cerebral arteries, it has been suggested that cilostazol is still effective under conditions with possible dysfunctional NO-cGMP pathway, such as in ischemic stroke or cerebral vasospasm [197]. Thus, it is of interest whether this drug could also have therapeutic potential in patients with cerebrovascular disorders.

In a previous report [185], it could be demonstrated that cilostazol prevented the progression of carotid intima thickness in patients with Type II diabetes mellitus. The group without cilostazol had a significant increase in infarct-like lesions which was positively correlated with the intima media thickness. The intima media thickness of the carotid artery is used as a surrogate of definite atherosclerosis with a high risk of vascular events [198–200]. The results of a secondary prevention study using cilostazol 200 mg/day showed a reduction of stroke by 43.3% compared with the placebo group [201]. However, cilostazol was compared with placebo without administering a standard antiplatelet therapy. Indeed, as this was a secondary prevention study of patients ( $n = 1052$ ) who suffered from cerebral infarction 1 to 6 months prior to enrolment, this appears inadequate. As the place of aspirin in the secondary prevention of stroke/TIA has been established to the satisfaction of most authors [39,164,165,202], the design of this study has to be criticised. Moreover, the antithrombotic effects of cilostazol after stent implantation may be somewhat overstated, particularly with drug-eluting stents. Indeed, patients receiving cilostazol had more acute/subacute stent thrombosis compared with those receiving clopidogrel.

Taken together, cilostazol has been routinely used as an antithrombotic agent for the treatment of peripheral arterial occlusive disease in Japan and some Asian countries for more than 15 years [203,204] and a new indication for stroke prevention has been recently approved in Japan [204]. In contrast, cilostazol has been available for the treatment of intermittent claudication in the USA since 1999 and in the UK since 2000 [204] but is not generally considered an antithrombotic agent in Western countries, perhaps due to the bulk of its antithrombotic preclinical and clinical development being conducted in Japan.

#### *Phosphodiesterase inhibitors in perspective*

NSP-513 is a novel selective PDE-III inhibitor on PDE isozyme activities and *in vitro* platelet aggregation and *in vivo* thrombus formation were investigated as reported recently [205]. NSP-513 selectively inhibited human platelet PDE-III isozyme *in vitro*. In a mouse pulmonary thromboembolism model, orally administered NSP-513 showed *in vivo* antithrombotic effects that were 320- to 470-times more potent than those of cilostazol. In a rat carotid arterial thrombosis model, intraduodenally administered NSP-513, cilostazol and aspirin reduced thrombus formation by 75, 66 and 48%, respectively. In contrast, intravenously administered dipyridamole did not significantly prevent thrombus formation and therefore NSP-513 is suggested to have the potential to prevent, not only *in vitro* platelet aggregation, but also *in vivo* thrombus formation. Of interest, this study shows that the antiplatelet and antithrombotic activities of NSP-513 are greater than those of cilostazol, dipyridamole or aspirin, thus, may have therapeutic potential in the treatment of arterial thrombotic disorders.

KW-7, a new inhibitor of cyclic nucleotide PDE, was shown to inhibit cAMP- and cGMP-PDE activities as well as AA-stimulated TXA<sub>2</sub> production [206]. This was associated with an increase in PGD<sub>2</sub> levels, indicating that KW-7 is also an inhibitor of TX synthase. In a very recent study, it was demonstrated that cilostazol and dipyridamole synergistically inhibited platelet aggregation *in vitro* and *ex vivo*, compared with treatment with either drug alone [207]. Although the dual inhibition of KW-7 on PDE and TX synthase and the combination of cilostazol and dipyridamole might provide an attractive target in developing new antiplatelet drugs, only few data exist and no data on the use of such inhibitors in clinical trials are currently available.

#### *Agents interfering with ADP-mediated platelet reactions*

##### *Thienopyridines (ticlopidine & clopidogrel)*

ADP is an important platelet agonist, which activates platelets by binding to purinergic receptors on the platelet surface. There are three recognized subtypes of P2 receptors on platelet membranes, namely P2X<sub>1</sub>, Y<sub>1</sub> and Y<sub>12</sub> [208–210]. The latter is the target of the antiplatelet thienopyridines, ticlopidine and clopidogrel [211,212]. Both drugs selectively inhibit ADP-induced platelet aggregation with no direct effects on AA metabolism (Figure 1) [213]. While not studied as extensively as aspirin, several clinical trials have confirmed the ability of thienopyridines to reduce cardiovascular events in patients with several different types of cardiovascular disease (Table 5) [214,215]. Moreover, ticlopidine has been established as an alternative to aspirin in the prevention of recurrent cerebral ischemia and stroke [216] but its use has been limited due to potentially detrimental side effects, including fatal severe neutropenia and thrombotic thrombocytopenic purpura [217] as well as aplastic anemia [218]. As a result, it has been replaced by clopidogrel [62,214,219]. Indeed, clopidogrel has become a mainstay in antiplatelet therapy [99,220,221] and several studies have been preformed using this drug (Table 5).

##### *Clinical studies of patients with vascular diseases, particularly patients with cardiovascular diseases*

The Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial, involving 19185 subjects, was the first randomized, double-blinded, international trial to evaluate the efficacy of aspirin 325 mg once daily versus clopidogrel 75 mg once daily in patients with recent ischemic stroke or MI, or established peripheral disease [214]. An intent-to-treat analysis of all randomized patients showed a modest 8.7% RRR ( $p = 0.043$ ) in the primary outcome of stroke, MI or vascular death. Interestingly, in a subgroup analysis, patients with peripheral arterial disease derived the greatest benefit from the drug (RRR: 23.8%;  $p = 0.0028$ ) whereas a nonsignificant 7.3% RRR in patients with stroke and a nonsignificant 3.7% risk increase in the primary outcome of patients with MI was obtained. Overall, the safety and tolerability of clopidogrel and aspirin were similar and, therefore, clopidogrel was established as an alternative antiplatelet to aspirin for secondary prevention across a wide spectrum of patients with vascular disease.

Table 5. Clinical studies of dual antiplatelet therapy with clopidogrel

Trial	Patients	Subjects (n)	Treatment	Primary end point	Result	Ref.
CLASSICS	Patients after coronary stenting	1020	Clopidogrel loading dose 300 mg + 325 mg aspirin, followed by clopidogrel 75 mg/day + aspirin 325 mg/day vs. clopidogrel 75 mg/day + aspirin 325 mg/day vs. ticlopidine 250 mg twice daily + aspirin 325 mg/day	Major peripheral or bleeding complications, neutropenia, thrombocytopenia or early discontinuation of study drug as the result of noncardiac adverse event during the study treatment period	50% RRR in the occurrence of primary endpoint in favor of clopidogrel (p = 0.005)	[219]
CREDO	Patients undergoing PCI	2116	Clopidogrel loading dose 300 mg ( <i>Group A</i> ) or placebo ( <i>Group B</i> ) before PCI plus aspirin 325 mg/day ( <i>Group A</i> + <i>Group B</i> ), followed by <i>Group A</i> : clopidogrel 75 mg/day + aspirin 325 mg/day for 12 months vs. <i>Group B</i> : clopidogrel 75mg/day for 28 days; from day 29 through 12 months placebo + aspirin 325 mg/day for 12 months	1-year incidence of the composite of death, MI or stroke in the intent-to-treat population and 28-day incidence of the composite of death, MI, or urgent target-vessel revascularization in the per-protocol population	Following PCI, long-term clopidogrel therapy significantly reduced the risk of ischemic events (27% RRR; p = 0.02) A loading dose of clopidogrel given before PCI did not reduce ischemic events at 28 days (18.5% RRR; p = 0.23). However, a subgroup of patients receiving clopidogrel >6 h before PCI experienced a 38.6% RRR (p = 0.051) compared with no reduction in patients receiving clopidogrel less than 6 h before PCI.	[224]
CURE	Unstable angina or non-ST segment elevation MI	12562	Clopidogrel loading dose 300 mg, followed by clopidogrel 75mg/day + aspirin 325 mg/day, vs. placebo loading dose, followed by placebo + aspirin 325 mg/day	Composite of cardiovascular death, MI or stroke and the composite of cardiovascular death, MI, stroke or refractory ischemia	20% RRR in ischemic events in favour of clopidogrel (p < 0.001) There were significantly more patients with major bleeding in the clopidogrel group than in the placebo group (3.7 vs 2.7%, 1.38 RRR; p < 0.001) but there were not significantly more patients with episodes of life-threatening bleeding (2.1 vs 1.8%; p = 0.13) or hemorrhagic strokes.	[222]

CREDO: Clopidogrel for Reduction of Events During Observation trial; CURE: Clopidogrel in Unstable angina to prevent Recurrent Events trial; MATCH: Management of Atherothrombosis with clopidogrel in high-risk patients with recent Transient isChemic attacks or Ischemic stroke trial; MI: Myocardial infarction; PCI: Percutaneous coronary intervention; PCI-CURE: Percutaneous coronary intervention – Clopidogrel in Unstable angina to prevent Recurrent Events trial; RRR: Relative risk reduction.

**Table 5. Clinical studies of dual antiplatelet therapy with clopidogrel (Cont.).**

Trial	Patients	Subjects (n)	Treatment	Primary end point	Result	Ref.
PCI-CURE	Subset of CURE undergoing PCI	2658	Clopidogrel loading dose 300 mg, followed by clopidogrel 75 mg/day + aspirin 325 mg/day vs placebo loading dose, followed by placebo + aspirin 325 mg/day. After PCI, patients received either clopidogrel or ticlopidine + aspirin for 2 to 4 weeks, after which administration of the randomly assigned study medication was started until the end of the scheduled follow-up	Composite of cardiovascular death, MI or urgent target-vessel revascularization within 30 days of PCI	30% RRR in ischemic events from PCI to 30 days in favor of clopidogrel ( $p = 0.03$ ). Overall (including ischemic events before and after PCI) there was a 31% RRR in cardiovascular death or MI ( $p = 0.002$ )	[70]
MATCH	Recent ischemic stroke or transient ischemic attack with at least one additional vascular risk factor	7599	Clopidogrel 75 mg/day + aspirin 75 mg/day vs clopidogrel 75 mg/day + placebo	First reoccurrence of ischemic stroke, MI, vascular death or rehospitalisation for an acute ischemic event	There was a nonsignificant 6.4% RRR in the primary endpoint when adding aspirin to clopidogrel ( $p = 0.244$ ). Adding aspirin to clopidogrel was associated with increased life-threatening bleedings (absolute risk increase 1.26%; $p < 0.0001$ ) and increased major bleeding (absolute risk increase 1.36%; $p < 0.0001$ ). No difference between groups was recorded in mortality.	[225]

CREDO: Clopidogrel for Reduction of Events During Observation trial; CURE: Clopidogrel in Unstable angina to prevent Recurrent Events trial; MATCH: Management of Atherothrombosis with clopidogrel in high-risk patients with recent Transient isChemic attacks or ischemic stroke trial; MI: Myocardial infarction; PCI: Percutaneous coronary intervention; PCI-CURE: Percutaneous coronary intervention – Clopidogrel in Unstable angina to prevent Recurrent Events trial; RRR: Relative risk reduction.

Moreover, CLASSICS demonstrated the superior efficacy and safety of clopidogrel plus aspirin compared with ticlopidine plus aspirin in patients undergoing coronary stenting [219]. This was the first randomized trial of clopidogrel in coronary stenting and the first study to evaluate clopidogrel–aspirin combination therapy and a loading dose of clopidogrel.

The CURE trial investigated the effect of clopidogrel combined with aspirin in the treatment of patients with acute coronary syndromes (ACSs) that included unstable angina and MI without ST-segment elevation [222]. A total of 12562 patients presenting within 24 h after the onset of symptoms received clopidogrel (300 mg immediately, followed by 75 mg once daily) or placebo in addition to aspirin for 3 to 12 months.

There was a RRR of 20% in (9.3% patients in the clopidogrel group vs. 11.4% in the placebo group;  $p < 0.001$ ) in the composite primary outcome of death from cardiovascular causes, non-fatal MI or stroke. The rates of bleeding were higher in the clopidogrel group than in the placebo group (3.7 vs. 2.7%;  $p = 0.001$ ), but there were not significantly more episodes of life-threatening bleeding. The PCI-CURE, a sub-study of CURE, showed that the benefit of clopidogrel over placebo was also seen in patients receiving PCI [70]. Overall, there was a 31% reduction in cardiovascular death or MI ( $p = 0.002$ ). Moreover, a meta-analysis of ten studies, comparing clopidogrel plus aspirin versus ticlopidine plus aspirin after coronary stenting demonstrated that clopidogrel, in addition to

better tolerability and fewer side effects, is at least as effective as ticlopidine in reducing 30-day major adverse cardiac events [223]. Thus, clopidogrel plus aspirin should replace ticlopidine plus aspirin as the standard antiplatelet regimen after stent deployment. Indeed, the combination of aspirin and clopidogrel has become standard treatment up to 12 months after coronary stent implantation [99].

The CREDO trial was designed to evaluate the benefit of clopidogrel pretreatment and long-term therapy to a more stable population undergoing coronary stenting [224]. Patients receiving 1 year instead of 1 month of clopidogrel showed a significant 27% RRR in the composite of death, MI or stroke. Clopidogrel pretreatment did not significantly reduce the combined risk of death, MI or urgent target vessel revascularization at 28 days. However, in a subgroup analysis, patients who received a loading dose of 300 mg at least 6 h before PCI experienced a 38.6% RRR ( $p = 0.051$ ) for this end point compared with no reduction with treatment less than 6 h before percutaneous intervention. Risk of major bleeding at 1 year increased, but not significantly.

To summarize, the findings of these randomized controlled trials have shown the sustained benefit of clopidogrel in addition to standard treatment including aspirin, especially in patients with coronary manifestation of atherothrombosis. The overall safety profile of clopidogrel is at least as good as that of medium-dose aspirin and is superior to that of ticlopidine.

*Clinical studies addressed to patients with cerebrovascular diseases or heart failure*

Data from the MATCH trial have recently been published [225]. The trial, involving 7599 patients, was a first randomized, double-blinded trial that was designed to assess whether the addition of aspirin 75 mg once daily to clopidogrel 75 mg once daily could have a greater benefit than clopidogrel alone in reducing the risk of recurrent ischemic vascular events in high-risk patients after TIA or ischemic stroke. Patients were included if they had an ischemic stroke or TIA in the previous 3 months and had one or more of five additional risk factors:

- Previous ischemic stroke
- Previous MI
- Angina pectoris
- Diabetes mellitus
- Symptomatic peripheral arterial disease

The results of this study are of interest, as the RRR in a subgroup analysis for stroke alone was not significant in the CAPRIE study [214]. However, the CAPRIE study was not designed to specifically address patients who had cerebrovascular disease.

The MATCH trial showed a nonsignificant RRR of 6.4% (16% patients in the clopidogrel and aspirin arm vs. 17% patients in the clopidogrel-only arm;  $p = 0.244$ ) in the composite primary outcome of ischemic stroke, MI, vascular death, or rehospitalization for an acute ischemic event. The 6.4% RRR in favor of aspirin plus clopidogrel in the intent-to-treat-analysis among all randomized patients is in the range that was reported about the stroke subgroup population (7.3%) in the CAPRIE study [214]. The rates of life-threatening bleeding were higher in the clopidogrel plus aspirin arm versus the clopidogrel-only arm (3 vs. 1%;  $p < 0.0001$ ) but in both treatment arms, no hemorrhagic transformation of ischemic stroke was reported as life-threatening bleeding and no significant difference was recorded in the incidence of fatal bleeding. However, several cardiology trials [70,222,224] have demonstrated a clear benefit of the combination of clopidogrel and aspirin over aspirin alone for the prevention of vascular end points in patients with coronary heart disease. Moreover, the increase in bleeding risk with the combination was smaller than in MATCH.

The differences between the MATCH trial and the cardiology trials could be due to different designs. Whereas in the cardiology trials clopidogrel was added to treatment, in the MATCH trial aspirin was added to clopidogrel. Consequently, the MATCH trial provided a measure of the benefit-to-risk ratio of aspirin in addition to clopidogrel, not for clopidogrel added to aspirin as in the cardiology trials. One major limitation of this study is that clopidogrel was used as the standard therapy for patients with cerebrovascular diseases although aspirin presently is considered the treatment of choice for secondary prevention of disorders associated with arterial thrombosis [39,164,165]. Indeed, a meta-analysis of more than 287 clinical trials showed that, overall, aspirin reduces the risk of stroke, MI and vascular death by approximately 23% in patients with various cardiovascular and cerebrovascular diseases [39]. Thus, recommendations for clopidogrel are usually made to patients who are intolerant of aspirin, who have had a recurrent ischemic event while on aspirin, or who are at vascular high risk [39,65,99,162,163,202,220].

In summary, the outcome of the MATCH trial indicates that the combination therapy of aspirin plus clopidogrel is not superior to clopidogrel alone, and that the addition of aspirin to clopidogrel results in significantly higher bleeding rates. As a consequence, the combination of aspirin and clopidogrel for cerebrovascular prevention should only be given within controlled studies. Indeed, the design of the PRoFESS trial has been changed [402] following the announcement of the results of the MATCH trial and this trial is no longer utilizing a combination of clopidogrel plus aspirin as the comparator – it is now clopidogrel alone. Unfortunately, whether clopidogrel is superior to aspirin in the treatment of cerebrovascular diseases cannot be concluded from the MATCH trial and therefore no recommendation can yet be given for the primary use of clopidogrel in these patients. Additional information could be obtained by a further study comparing clopidogrel versus aspirin addressed especially to patients with cerebrovascular diseases. Indeed, this is of interest as several controversial recommendations and guidelines concerning the use of antiplatelet drugs in patients with TIA or stroke have been published [162–165,202,226–228] but there are no data available regarding how these recommendations translate into clinical practice and which affect the choice of antiplatelet drugs in patients with a recent ischemic cerebrovascular event.

The Warfarin and Antiplatelet Therapy in Chronic Heart failure (WATCH) trial was designed to determine the optimal antiplatelet/thrombotic agent for heart failure. Patients were randomized to open-label warfarin (target international normalized ratio [INR]: 2.5–3.0) or double-blind antiplatelet therapy with aspirin 162 or clopidogrel 75 mg. Unfortunately, the trial had to be terminated after a study period of 18 months due to poor enrolment, with a resulting reduction of its power to achieve its original objectives [229].

#### *Thienopyridines in perspective*

Thienopyridines have become a mainstay in antiplatelet therapy. Ticlopidine is currently used as a reserve drug due to its unfavourable side-effect profile (neutropenia and thrombocytopenic purpura) and has been replaced by clopidogrel. Clopidogrel has been shown to be superior to aspirin in patients with atherothrombotic disease [214]. The benefit of clopidogrel appears particularly pronounced in patients with diabetes, prior revascularization, and

prior ischemic events [230–232]. Clopidogrel, in combination with aspirin, has been demonstrated to be more efficacious than aspirin alone in patients presenting with ACS, with a favorable safety profile [222,224,233].

As most of the trials were addressed to patients with cardiovascular diseases, numerous trials have now been initiated to study the benefits of combining aspirin and clopidogrel for other indications such as stroke or atrial fibrillation (Table 6). One of these interesting studies is the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) study (Table 6) which was designed to evaluate the efficacy and safety of clopidogrel plus aspirin versus placebo plus aspirin in patients with established coronary, cerebral or peripheral arterial disease or in patients with multiple risk factors for atherothrombosis who have not yet suffered from an ischemic event [234]. The results of this study will be of interest since it is unknown whether dual antiplatelet therapy is superior to aspirin monotherapy for high-risk primary and secondary prevention. This large-scale trial of patients at a high risk for atherothrombotic events will allow determination of the value of the strategy of adding clopidogrel to the current standard of care, including low-dose aspirin, for a wide spectrum of patients with atherothrombosis.

#### *Clopidogrel: a better antiplatelet drug for the prevention of gastrointestinal bleeding?*

An overview of randomized trials of aspirin therapy found that GI toxicity (both major and minor) was dose related, with daily doses between 30 and 1300 mg [235]. Even when administered at very low doses (30–50 mg/day), aspirin can cause serious GI bleeding [69,159]. Proton-pump inhibitors reduce the risk of aspirin-induced ulcera-bleeding [236,237] and thus, concurrent therapy with these drugs has become a standard treatment for patients at risk for ulcera bleeding who are taking aspirin [238,239].

An alternative strategy is to replace aspirin with another antiplatelet drug that does not induce GI ulcera. Clopidogrel has been shown to be potentially superior to aspirin in patients with atherothrombotic disease [214]. Moreover, administration of clopidogrel resulted in a slightly lower rate of GI bleeding when compared with aspirin (0.5 vs. 0.7%) [214] and did not induce gastric damage in healthy volunteers [240]. Thus, clopidogrel has recently been recommended for patients unable to take



**Table 6. Ongoing trials with clopidogrel.**

Trial	Patients	Number of subjects	Study design
ACTIVE A	Atrial fibrillation	7500	Patients with a contraindication to warfarin or who refuse anticoagulant therapy will be randomized to receive either clopidogrel 75 mg/day + aspirin 75–100 mg/day or aspirin 75–100 mg/day
ACTIVE W	Atrial fibrillation	6500	Patients eligible for anticoagulants will be randomized to receive either clopidogrel 75 mg/day + aspirin 75–100 mg/day or adjusted dose vitamin K antagonist (INR 2.0–3.0)
ARCH	Aortic arch atheroma	1500	Patients will be randomized to receive either warfarin (INR 2.0–3.0) or aspirin 75–325 mg/day + clopidogrel 75 mg/day
CAMPER	Peripheral arterial intervention	2000	Unknown
CASPAR	Peripheral arterial bypass surgery	1460	Unknown
CCS-2/COMMIT	Acute ST-segment elevation MI	45000	Clopidogrel 75 mg/day + aspirin versus aspirin alone
CHARISMA	Secondary and high-risk primary prevention	15603	Two treatment groups will be randomized to receive either clopidogrel 75 mg/day or placebo, in combination with aspirin 75–162 mg/day
CLARITY	Acute ST-segment elevation MI	3000	Clopidogrel 75mg/day + aspirin vs aspirin alone
FASTER	Acute TIA and minor ischemic stroke within 12 hours of onset	7500	All patients will be on aspirin. Patients will be randomized to clopidogrel (300 mg loading dose + 75 mg/day) or placebo, and to simvastatin (40 mg/day) or placebo
PRoFESS	Secondary prevention of stroke	15500	Patients will be randomized to either of two antiplatelet combination regimens: aspirin/ERDP (25 mg aspirin/200 mg extended-release dipyridamole) or clopidogrel 75 mg/d, and randomized to treatment with telmisartan 80 mg or placebo
SPS3	Secondary prevention of stroke, major vascular events and cognitive decline among patients with small subcortical stroke	2500	Patients will be randomized to receive either aspirin 325 mg/day or a combination of aspirin 325 mg/day + clopidogrel 75 mg/day. Hypertensive S3 patients will be randomized to receive antihypertensive therapy with a target systolic blood pressure of either <150 mmHg or <130 mmHg.

*ACTIVE: Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events A trial; ARCH: Aortic arch Related Cerebral Hazard; CAMPER: Clopidogrel and Aspirin in the Management of Peripheral Endovascular Revascularization; CASPAR: Clopidogrel and Aspirin in bypass Surgery for Peripheral ARterial disease; CCS2/COMMIT: Second Chinese Cardiac Study/Clopidogrel and Metoprolol Myocardial Infarction Trial; CHARISMA: Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance; CLARITY: Clopidogrel as Adjunctive Reperfusion Therapy; FASTER: Fast Assessment of Stroke and TIA to prevent Early Recurrence study INR: International normalized ratio; MI: Myocardial infarction; PRoFESS: Prevention Regimen For Effectively avoiding Second Strokes trial; SPS3: Secondary Prevention of Stroke 3 trial; TIA: Transient ischemic attack.*

aspirin owing to previous GI intolerance [65,220]. However, although one study found a lower incidence of GI bleeding among patients receiving clopidogrel than in those receiving aspirin, a relatively high dose of aspirin (325 mg/day) was used for the secondary prevention of cardiovascular/cerebrovascular events in this study [214].

Interestingly, until very recently, there has been no prospective trial available to assess whether clopidogrel is an alternative to aspirin plus a proton-pump inhibitor for patients at risk for ulcers. Currently, the results of a study in

which clopidogrel (75 mg/day) was compared with aspirin (80 mg/day) plus esomeprazole (20 mg twice daily) in high-risk patients who had a history of aspirin-induced upper GI bleeding, have been reported [241]. Over a 1-year follow-up period, the incidence of recurrent ulcers bleeding was significantly higher in patients taking clopidogrel when compared with those taking aspirin plus esomeprazole (8.6 vs. 0.7%). Among patients with a history of aspirin-induced ulcers bleeding, aspirin plus esomeprazole was definitely superior to clopidogrel for the prevention of recurrent GI bleeding.

At present the current recommendation for patients who already have had a GI complication while taking aspirin is to replace aspirin with clopidogrel [39,65,99,220]. However, although there are several potential limitations of the recently published study [241,242], the observation of this study clearly do not support the current recommendation that clopidogrel should be used for patients who already have had GI complications while taking aspirin.

*Development of new drugs interfering with ADP-mediated platelet reaction*

The development of new ADP-receptor antagonists possessing high potency and platelet selectivity provides a new pharmacologic approach for modulating platelet reactivity with the potential to prevent arterial thrombosis.

A more recent and related P2Y<sub>12</sub> receptor antagonists, AR-C69931MX [243], is found to be a highly potent and selective antagonist at the P2Y<sub>12</sub> receptor that, unlike clopidogrel, is active *in vitro* [243–245]. AR-C69931MX reversibly inhibits aggregation, granule secretion, P-selectin expression and procoagulant activity induced by agonists other than ADP, including U46619, thrombin receptor-activating peptide and collagen [244,246]. In a canine model, AR-C69931MX administered as an intravenous infusion (4.0 µg/kg/min) was shown to prevent occlusive arterial thrombus formation *in vivo* in response to a deep arterial lesion in the canine carotid artery [247]. The first Phase II study of intravenous AR-C69931MX in patients with ACS showed that it was well tolerated as adjunctive therapy, including heparin and aspirin, with effective inhibition of ADP-induced platelet aggregation, rapid onset of action and a plasma half-life of only several minutes [245]. Moreover, it was demonstrated that, in both healthy volunteers and patients with ACS, AR-C69931MX inhibited ADP-induced platelet aggregation, P-selectin expression and platelet-leukocyte conjugate formation *in vitro* and *in vivo*, whereas aspirin had no effect on any of these responses [248].

Another novel thienopyridine P2Y<sub>12</sub> receptor antagonist is CS-747 [249]. CS-747 inactivates the P2Y<sub>12</sub> receptor through its active metabolite, by the same mechanism such as clopidogrel [249–251]. Currently, CS-747 (prasugrel) is being investigated for the treatment of patients with ACS who undergo PCI. The Phase IIb Joint Utilization of Medications to Block platelets Optimally – Thrombolysis In Myocardial Infarction (JUMBO-TIMI) 26 trial, presented at

the European Society of Cardiology (ESC) in August 2004, evaluated 904 patients. Subjects received aspirin 325 mg daily and were assigned to either clopidogrel (300-mg loading dose and 75 mg maintenance doses for 1 month) or one of three prasugrel loading- and maintenance-dose regimens [403]. There was no significant difference in bleeding between combined prasugrel groups and clopidogrel (1.7% prasugrel vs. 1.2% clopidogrel; *p* = 0.77). Death, MI, stroke, clinical target vessel thrombosis and severe recurrent ischemia nonsignificantly favored prasugrel (7.2% prasugrel vs. 9.4% clopidogrel; *p* = 0.31) without a dose–response effect [252]. Despite limitations, predominantly due to the exploratory nature of the trial, the results are interesting and promising and clinically significant differences in efficacy have to be established in the future by the Triton TIMI-38 trial. Patients will be followed on maintenance therapy for 12 months. In this trial, 13,000 patients with ACS will be enrolled and randomized to either prasugrel or standard doses of clopidogrel. The primary end point will be the composite of cardiovascular death, MI, and stroke. The secondary end point will include bleeding, recurrent ischemia and urgent target-vessel revascularization [403].

Since it is known that P2Y<sub>1</sub>-null mice display strong resistance to the thromboembolism induced by intravenous injection of ADP, a mixture of collagen and adrenalin [253,254] and thromboplastin [255] the P2Y<sub>1</sub> receptor represents a potential pharmacological target for anti-thrombotic drugs. Indeed, the administration of the reversible P2Y<sub>1</sub> receptor antagonist MRS2179 to mice was shown to strongly inhibit ADP-induced aggregation [253,255,256]. As both P2Y<sub>1</sub> and Y<sub>12</sub> antagonists alone can potently inhibit ADP-induced platelet activation [257,258] it was of interest to investigate whether there is a synergistic effect of these antagonists. In a recently published study a synergistic effect regarding inhibition of ADP-induced platelet activation was obtained with the combination of the P2Y<sub>12</sub> antagonist AR-C69931MX and the P2Y<sub>1</sub> antagonist MRS2179 [259]. Moreover, a strong synergistic effect in inhibition of thrombin-induced platelet activation with combination of AR-C69931MX and the thrombin inhibitor melagatran could also be shown. Whether the synergistic effect *in vitro* also results in an improved antithrombotic effect *in vivo* with or without an increased risk of bleeding should be established in further studies.

Another promising target of ADP-receptor antagonists could be the P2X<sub>1</sub> receptor. An excellent review has recently been published focusing on the role of P2X<sub>1</sub> receptors in platelet activation [210]. Indeed, results from functional genomic studies showed that P2X<sub>1</sub> receptors significantly increased the risk of thrombosis whereas knockout of P2X<sub>1</sub> receptors led to a reduced risk of thrombosis [260]. Moreover, it has been suggested from both knock-in and -out studies, that this receptor appears to contribute to platelet activation under condition of high shear rates such as found in the arterial circulation [210].

To summarize, the ATP analogs of the AR-C series, which are potent competitive ADP-receptor antagonists, have proved to be efficient platelet drugs in animal models and some of them are in Phase II clinical trials for the treatment of ACS. Moreover, in addition to the clopidogrel or the AR-C compound-sensitive P2Y<sub>12</sub> receptor, the P2Y<sub>1</sub> and X<sub>1</sub> receptor are promising potential targets for new antithrombotic drugs.

#### *GPIIb/IIIa receptor antagonists*

It is well established that the expression of GPIIb/IIIa on the platelet surface is the final common pathway of platelet aggregation [261]. These receptors recognise an arginine-glycine-aspartic-acid sequence contained in adhesive molecules such as fibrinogen and von Willebrand factor (vWF). When platelets get activated, GPIIb/IIIa is converted into a functional receptor, binding these proteins and allowing platelets to aggregate and form a hemostatic plug [8]. Thus, this receptor has become the target of novel antiplatelet drugs [262]. Murine monoclonal antibodies were the first antagonists of the GPIIb/IIIa receptor to be developed [263]. Platelet aggregation is highly inhibited by the blockade of 80% of the surface GPIIb/IIIa receptors [264]. Three classes of GPIIb/IIIa antagonists have been developed [265]:

- Murine–human chimeric antibodies, such as abciximab
- Synergistic peptide forms, such as eptifibatide
- Synthetic nonpeptide forms, such as tirofiban and lamifiban

The development of this new class of drugs that blocks fibrinogen binding to the GPIIb/IIIa receptors (Figure 1) has raised the possibility that these potent agents may reduce thrombotic complications in ACS or after PCI. A large number of trials have been carried out so far and an excellent review on the current status of antiplatelet

therapy with GPIIb/IIIa antagonists has recently been published [266]. Recommendations for the use of GPIIb/IIIa antagonists in patients with cardiovascular diseases are available from evidenced-based guidelines [99,221,220].

#### *GPIIb/IIIa antagonists in cardiovascular disease*

##### *Intravenous GPIIb/IIIa antagonists for PCI in high-risk patients and in patients with ACS without persistent ST-segment elevation*

As previously reported, there is outstanding evidence supporting the utility of intravenous GPIIb/IIIa inhibition, mainly for abciximab, as an adjunct to aspirin and heparin in high-risk patients undergoing PCI and in patients with ACS without persistent ST-segment elevation before revascularization [266]. Indeed, the use of GPIIb/IIIa antagonists has been validated in dedicated trials for patients undergoing PCI with or without stenting [267–271]. However, a recent clinical trial was designed to evaluate the possible additional benefit of the GPIIb/IIIa antagonist abciximab in 2159 patients at low-to-intermediate risk undergoing PCI pretreated with 600 mg of clopidogrel [272]. The trial showed no additional benefit of abciximab in the composite primary outcome of death, MI or target-vessel revascularization within the first 30 days in those patients. Moreover, the role in the purely medical management of patients with ACS without persistent ST-segment elevation is less certain [266].

##### *Intravenous GPIIb/IIIa antagonists for*

##### *primary percutaneous transluminal coronary angioplasty (with or without stenting) in acute myocardial infarction*

On the basis of trials investigating the possible benefit of intravenous GPIIb/IIIa antagonists for primary PTCA in acute MI it is suggested that there is no definite indication for these drugs as an adjunct to primary PTCA, as this treatment modality was demonstrated to have variable effects [266]. Indeed, there had been debate in this field since the results of the four largest trials [273–276] did not support the benefit of abciximab at the primary end point timing of 6 months. Moreover, the study designs and methodology were quite variable in the clinical setting of these trials. Finally, the data on tirofiban and eptifibatide in primary PCI are far more limited than for abciximab [99,277]. Thus, given the size and limitations of the available data set, the routine administration of GPIIb/IIIa antagonists with PCI (with or without stenting) in patients with MI is still a matter of debate [266,278].

However, a meta-analysis of pooled data from the previous four trials [277] and of the more recent Abciximab and Carbostent Evaluation (ACE) trial (Table 7) [279] showed an overall 46% reduction in death, reinfarction and target vessel revascularization; a 34% reduction in death or reinfarction; and a 26% reduction in death at 30 days [277]. In all of the trials except the Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications trial (CADILLAC), there was support for abciximab treatment benefit for reduction of death or reinfarction at 6 months' follow-up, and in both Abciximab before Direct angioplasty and stenting in Myocardial Infarction Regarding Acute and Long-term follow-up (ADMIRAL) and ACE the differences were statistically significant (Table 7).

Moreover, concerning the effects of early versus delayed administration of GPIIb/IIIa antagonists in patients with ST-segment elevation MI undergoing PCI a meta-analysis of 6 randomized trials [283] showed that the early administration of abciximab or tirofiban appeared to improve

coronary patency with favorable trends for clinical outcomes. The early administration of GPIIb/IIIa antagonists was associated with a 28% relative reduction of mortality from 4.7 to 3.4%, which was not significant but consistent with similar trends for reinfarction and the composite ischemic end point.

To summarize, although there has been controversy in the routine administration of GPIIb/IIIa antagonists with PCI [277,278], it is now recommended that treatment with abciximab is started as early as possible in patients undergoing primary PCI (with or without stenting) [277,281] and treatment with tirofiban or eptifibatid may be considered before primary PCI (with or without stenting) [99]. Moreover, the meta-analysis by Topol and colleagues [277] clearly indicates that catheter-based reperfusion with adjunctive abciximab should be considered the preferred reperfusion therapy for acute MI and do not justify a different level of recommendation for the use of GPIIb/IIIa inhibitors in acute MI according to whether or not a stent is implanted.

**Table 7. Clinical studies with intravenous GPIIb/IIIa antagonists for PTCA in acute MI**

Trial	Patients	Subjects (n)	Treatment	Primary end point	Result	Ref.
RAPPORT	Acute MI within 12 h and ST-segment elevation in two contiguous leads or a new complete left bundle-branch block pattern, referred for coronary angioplasty	483	Abciximab 0.25 mg/kg bolus followed by a 12-h infusion of 0.125 µg/kg/min + stenting vs. placebo + stenting	Composite of death from any cause, nonfatal reinfarction, and any TVR within 6 months, or composite of death, reinfarction, and urgent TVR at 7 and 30 days.	No difference in primary 6-month endpoint, although abciximab significantly reduced death, reinfarction or urgent TVR at all time points measured. Major bleeding was significantly higher with abciximab than with placebo (16.6 vs. 9.5%; p = 0.02)	[273]
ISAR-2	Patients undergoing stenting within 48 h after onsets of symptoms of acute MI and ST-segment elevation in two or more contiguous leads	401	Abciximab 0.25 mg/kg bolus followed by 12-h infusion of 10 µg/min + reduced-dose heparin + stenting vs. standard dose heparin+ stenting	Composite of death, reinfarction, and TVR at 30 days	The primary 30-day end point was reached in 5.0% of the abciximab group and in 10.5% of the control group (p = 0.038). During 1-year follow-up, there was no additional benefit from a reduction in TVR nor did abciximab reduce angiographic restenosis.	[274]

ACE: Abciximab and Carbostent Evaluation trial; ACS: Acute coronary syndromes; ADMIRAL: Abciximab before Direct angioplasty and stenting in Myocardial Infarction Regarding Acute and Long-term follow-up trial; CADILLAC: Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications trial; ECG: Electrocardiography; ISAR: Intracoronary Stenting and Angiographic Results trial; MI: Myocardial infarction; PTCA: Percutaneous transluminal coronary angioplasty; RAPPORT: ReoPro And Primary PTCA Organization and Randomized Trial; TVR: Target vessel revascularization.

**Table 7. Clinical studies with intravenous GPIIb/IIIa antagonists for PTCA in acute MI (Cont.).**

Trial	Patients	Subjects (n)	Treatment	Primary end point	Result	Ref.
ADMIRAL	Patients undergoing stenting within 12 h after onset of	300	Abciximab 0.25 mg/kg bolus, followed by a 12-h infusion of 0.125 µg/kg/min + stenting vs. placebo + stenting	Composite of death, reinfarction, or urgent TVR at 30 days	Significant difference in primary 30-day endpoint (6% for abciximab and 14.6% for placebo; p = 0.01) which remained significant through 6-month follow up (7.4 vs. 15.9%; p=0.02) One major bleeding event occurred in the abciximab group compared to none in the placebo group The primary 6-month endpoint occurred in 20.0% after PTCA, 16.5% after PTCA + abciximab, 11.5 % after stenting, and 10.2% after stenting + abciximab (p < 0.001)	[275]
CADILLAC	symptoms of acute MI and ST-segment elevation in two contiguous leads Symptoms of MI >30min within 12 h and lytic eligible ECG	2082	Abciximab + PTCA vs. PTCA alone vs. abciximab + multiLink stent vs. multiLink stent alone	Composite of death, reinfarction, disabling stroke, and ischemia-driven revascularization of the target vessel at 6 months	No differences among the groups in the rates of death, stroke or reinfarction, although abciximab did reduce rates of repeated revascularization during the first week postinitial procedure. The primary 30-day endpoint occurred in 10.5% after stenting, and 4.5% after abciximab + stenting (p = 0.023)	[276]
ACE	Symptoms of MI >30 min associated with ST-segment elevation in two or more contiguous leads within 6 h or between 6 and 24 h if there was evidence of continuing ischemia. Patients with cardiogenic shock due to predominant ventricular failure were also included	400	Abciximab 0.25 mg/kg bolus, followed by a 12-h infusion of 0.125 µg/kg/min + stenting vs. stenting alone	Composite of death from any cause, reinfarction, TVR, and stroke at 30 days	At 6 months, the cumulative difference in mortality between the groups increased (4.5 vs. 8%), and the incidence of the composite of 6-month death and reinfarction was lower in the abciximab group than in the stent only group (5.5% and 13.5%, p = 0.006). Six-month repeat TVR and restenosis rates were similar between the two groups.	[279]

ACE: Abciximab and Carbostent Evaluation trial; ACS: Acute coronary syndromes; ADMIRAL: Abciximab before Direct angioplasty and stenting in Myocardial Infarction Regarding Acute and Long-term follow-up trial; CADILLAC: Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications trial; ECG: Electrocardiography; ISAR: Intracoronary Stenting and Angiographic Results trial; MI: Myocardial infarction; PTCA: Percutaneous transluminal coronary angioplasty; RAPPORT: ReoPro And Primary PTCA Organization and Randomized Trial; TVR: Target vessel revascularization.

*Intravenous GPIIb/IIIa antagonists as adjuncts to lytic therapy in acute myocardial infarction*

The use of GPIIb/IIIa antagonists as adjuncts to lytic therapy in acute MI is not recommended [266] as the results of the large GUSTO V study were disappointing with no difference in mortality rates [282]. Indeed, the clinical efficacy of GPIIb/IIIa antagonists has been equivocal in terms of mortality in patients with ST-elevated MI, although a subgroup analysis of the ADMIRAL trial has suggested that the early administration of abciximab may provide further benefit [280]. A meta-analysis of 16 randomized, controlled trials demonstrated that the parenteral GPIIb/IIIa antagonists significantly reduced mortality at 48 to 96 h but mortality benefits of 30 days and 6 months were not statistically significant [284]. However, the meta-analysis showed that GPIIb/IIIa antagonists provided a consistent and sustained therapeutic benefit on death, MI, and revascularization in patients with ischemic heart disease.

*Limitations of intravenous GPIIb/IIIa antagonists*

Although GPIIb/IIIa antagonists have been shown to be beneficial, the drugs have a narrow therapeutic window with regard to potential complications such as increased bleeding risk or thrombocytopenia particularly for abciximab [265]. However, across all clinical trials, the rate of life-threatening bleeding and intracranial haemorrhage was less than 0.2% and the most common bleeding site was the vascular access site [286]. Critical thrombocytopenia, eventually leading to uncontrolled bleeding, has been estimated in 0.4 to 1.6% patients treated with abciximab and severe thrombocytopenia does even occur in healthy volunteers [287]. In trials with other GPIIb/IIIa antagonists, the incidence of thrombocytopenia is generally lower than 1% [287]. Overall, intravenous GPIIb/IIIa antagonists have become a mainstay in the treatment of patients undergoing PCI and in these patients with ACS undergoing revascularization [266].

*GPIIb/IIIa antagonists in cerebrovascular disease*

Compared with coronary arterial diseases, only few trials have evaluated the efficacy and tolerability of platelet GPIIb/IIIa antagonists in patients with cerebrovascular diseases. On the basis of experience in ACS, parenteral GPIIb/IIIa antagonists may have potential applications in the treatment of acute ischemic stroke and as adjunctive therapy to carotid angioplasty. Indeed, the administration of agents such as

SM-20302 [288,289], ME3277 [290,291], murine 7E3 F(ab')<sub>2</sub> [292,293] and SDZ-GPI 562 [294] have been reported to preserve microvascular patency in different animal models of acute ischemic stroke and they may have neuroprotective properties. More recently, FK419, a novel nonpeptide GPIIb/IIIa antagonist dose-dependently shortened the time to first reperfusion and the total middle cerebral artery occlusion time and reduced ischemic brain damage in a guinea-pig model [295]. Thus, GPIIb/IIIa antagonists may be suitable as a single therapeutic or as an adjunct therapeutic to thrombolysis with alteplase for the treatment of stroke, although bleeding risk will be a major concern.

Results of pilot trials in the setting of acute ischemic stroke with the three GPIIb/IIIa antagonists abciximab, tirofiban, and eptifibatid are promising [288,296–299]. To evaluate the safety of abciximab in acute ischemic stroke a randomized, double-blind, placebo-controlled, dose-escalation trial was conducted in patients presenting within 24 h following ischemic stroke onset [300] who were randomized to receive either an escalating dose of abciximab or placebo. There were no identified cases of fatal or nonfatal major intracranial hemorrhage within either 5 days or 3 months of randomization. Asymptomatic parenchymal hemorrhages were detected on poststudy agent computed tomography in 7% of patients treated with abciximab and 5% of the placebo-treated patients. Overall, abciximab is considered safe when administered up to 24 h after stroke onset. Analysis of the pooled abciximab data provided some preliminary evidence that this agent might improve outcome after stroke, since the proportion of patients with minimal residual disability at 90 days was higher in the abciximab group.

The currently ongoing Safety of Tirofiban in acute Ischemic Stroke (SaTIS) trial, in which patients receive either tirofiban or placebo, will help to further evaluate the safety and efficacy of GPIIb/IIIa antagonists in the treatment of acute stroke [301]. Moreover, GPIIb/IIIa antagonists in combination with reduced doses of thrombolytic agents may have the potential for improving safety and efficacy compared with standard recombinant tissue plasminogen activator [298]. However, GPIIb/IIIa antagonists cannot be recommended for general use in patients with cerebrovascular disease before prospective randomized placebo-controlled clinical trials are completed.

**Table 8. Clinical studies with oral GPIIb/IIIa antagonists.**

Trial	Patients	Subjects (n)	Treatment	Primary end point	Result	Ref.
OPUS-TIMI 16	ACS within 72 h; history of cardiovascular disease, positive cardiac markers or ECG changes, peripheral or cerebrovascular disease, or DM	10302	Orbofiban 50 mg twice daily vs. orbofiban 50 mg twice daily for 30 days, followed by 30 mg twice daily vs. placebo	Death, MI, recurrent ischemia, urgent revascularisation or stroke	Trial terminated prematurely due to an unexpected increase in 30-day mortality in the orbofiban group Major or severe bleeding was higher with orbofiban; it occurred in 2.0%, 3.7% and 4.5% of patients	[302]
SYMPHONY	ACS after stabilisation	9233	Aspirin 80 mg twice daily vs. low-dose sibrافiban twice daily vs. high-dose sibrافiban twice daily	Death, MI, and severe recurrent ischemia at 90 days	No difference between aspirin group (9.8%), low- (10.1%), or high-dose sibrافiban (10.1%). Sibrافiban was associated with increased major bleeding	[303]
SYMPHONY-2	ACS after stabilisation	6671	Aspirin 80mg twice daily vs. low-dose sibrافiban plus aspirin 80 mg twice daily vs. high-dose sibrافiban twice daily	Death, MI and severe recurrent ischemia	No difference between aspirin (9.3%), low-dose sibrافiban plus aspirin (9.2%) or high-dose sibrافiban (10.5%) Aspirin with low-dose sibrافiban caused more bleeding than aspirin alone. There was a trend toward increased mortality in this group and a significant increase in the high-dose arm	[304]
EXCITE	Patients undergoing PCI	7232	Xemilofiban 20 mg or placebo before PCI, followed by Xemilofiban 10 mg three-times daily vs., xemilofiban 20 mg three-times daily vs. placebo	Death, MI and recurrent revascularization at 30 and 182 days	No difference between placebo (13.5%), 10 mg (13.9%) or 20 mg xemilofiban (12.7%) at 182 days. Significant increase in major bleeding in the xemilofiban group	[305]
BRAVO	Recent ACS, stroke, TIA, or peripheral vascular disease	9200	Lotrafiban 30 mg + aspirin twice daily vs. lotrafiban 50 mg+ aspirin twice daily vs. placebo	Death, MI, stroke, recurrent ischemia requiring hospitalization and urgent revascularization	Stopped at interim analysis because lotrafiban had a higher mortality than placebo (2.7 vs. 2.0%). Lotrafiban was associated with a higher mortality, more major bleeding, and a greater risk of serious thrombocytopenia	[306]

ACS: Acute coronary syndromes; BRAVO: Blockade of the glycoprotein IIb/IIIa Receptor to Avoid Vascular Occlusion trial; DM: Diabetes mellitus; ECG: Electrocardiography; EXCITE: Evaluation of oral Xemilofiban in Controlling Thrombotic Events; MI: Myocardial infarction; OPUS-TIMI: Orbofiban in Patients with Unstable coronary Syndromes – Thrombolysis In Myocardial Infarction; PCI: Percutaneous coronary intervention; SYMPHONY: Sibrافiban versus aspirin to Yield Maximum Protection from ischemic Heart Events post-acute Coronary syndromes trial; TIA: Transient ischemic attack.

#### Oral antagonists of GPIIb/IIIa receptors

To further test the clinical efficacy of GPIIb/IIIa antagonists, oral agents of this interesting class of drugs have been developed but only led to disappointing clinical results.

To date, five large Phase III trials including 45,523 patients investigated the clinical outcomes

of patients with ischemic heart disease treated with oral GPIIb/IIIa antagonists (Table 8) [302–306]. In contrast to the favorable results of the intravenous GPIIb/IIIa antagonists, the long-term use of oral GPIIb/IIIa antagonists showed no benefit in reducing major adverse cardiac events, and significantly increased mortality in these patients.

Indeed, a meta-analysis of trials with oral GPIIb/IIIa antagonists demonstrated a 31% increase in mortality rate [307]. Despite the lack of clinical benefit, excess bleeding complications were associated with the oral GPIIb/IIIa antagonists in these trials and, as a consequence, new oral drugs of this class were synthesized and tested in dose-response and safety studies.

The safety and tolerability of roxifiban, a second-generation oral nonpeptide platelet GPIIb/IIIa receptor antagonist, was tested in 98 patients with a history of chronic stable angina pectoris [308]. Roxifiban-induced inhibition of platelet aggregation was dose dependent and sustained throughout the study period: higher drug dosages correlated with higher levels of platelet inhibition and a higher incidence of minor bleeding events. No serious adverse events were observed at any dosage. However, the Roxifiban Oral Compound Kinetics Evaluation Trials (ROCKET)-I trial demonstrated that, despite achieving sustained inhibition of platelet aggregation, therapy with roxifiban was associated with over expression or phasic changes of major platelet receptors in patients with CAD [309]. Another second-generation oral platelet GPIIb/IIIa receptor antagonist is the prodrug UR-3216. UR-3216 maintains a high level of inhibition of platelet aggregation and, due to a small peak-to-trough ratio, severe bleeding is avoided [310]. Moreover, UR-3216 induces no prothrombotic activity in human platelets, distinctly different from orbofiban and other small molecule antagonists [311]. Although UR-3216 may be a promising orally-active GPIIb/IIIa antagonist, hardly any data on this drug are available.

To summarize, oral GPIIb/IIIa antagonists are not effective in reducing ischemic events when used on a long-term basis after ACS and, currently, it seems unlikely that second-generation oral platelet GPIIb/IIIa receptor antagonists will be brought into Phase III testing.

#### *Dark side of GPIIb/IIIa antagonists & future directions*

Paradoxical platelet activation after treatment with oral GPIIb/IIIa antagonists may be responsible for and explain the increased rate of mortality and high incidence of acute thrombotic events in patients with CAD. Moreover, the inability to maintain stable sustained platelet inhibition for chronic regimes contributes substantially to the withdrawal of this class of drugs.

An excellent review on antiplatelet therapy provides some possible explanations for this 'dark side' of GPIIb/IIIa antagonists [312]. In general, the disappointing results of the oral agents sibrifiban, orbofiban and xemilofiban may be due to suboptimal levels of platelet inhibition, leading to paradoxical agonist-induced platelet activation and/or vascular inflammation [312–315]. Indeed, it could be possible that the short half-life of oral GPIIb/IIIa antagonists function as antagonists at peak concentrations (causing bleeding) but at lower concentrations they might act as partial agonists via a mechanism known as 'platelet escape' [316,317]. Thus, these agents may cause bleeding at peak levels and promote thrombosis at trough levels in the same patient. Indeed, previous studies have demonstrated that chronic inhibition of platelets with oral GPIIb/IIIa antagonists might be associated with platelet receptor activation, continued procoagulant activity or enhanced platelet-neutrophil interaction [318–321]. Apart from that, even intravenous GPIIb/IIIa antagonists such as tirofiban have limited efficacy under conditions of platelet activation, high vWF release, high shear stress and physiological calcium concentrations [322].

In a more recent study, it has been demonstrated that the oral GPIIb/IIIa antagonist roxifiban significantly decreased ADP- and collagen-induced platelet aggregation, although platelet receptors such as GPIIb/IIIa, P-selectin, and platelet/endothelial cell-adhesion molecule (PECAM)-1 were paradoxically activated, monotherapy with aspirin resulted in a mild, but consistent, inhibition of these receptors. Moreover, oral GPIIb/IIIa antagonists may have proinflammatory potency at subtherapeutic levels, which could be an important factor in the toxicity of these agents [312].

Overall, various factors have been proposed to explain their failure, such as:

- Low affinity for the receptor
- Large peak-to-trough ratio
- Low bioavailability
- Partial agonist activity
- Proaggregatory effect
- The fact that oral GPIIb/IIIa antagonists don't seem to entice the clinical market

#### Monitoring of platelet function

Although the role of antiplatelet drugs in the treatment of vascular disease is well established, there remains concern about aspirin resistance [323], the less well known clopidogrel resistance [324], and nonresponsiveness to other



antiplatelet drugs such as GPIIb/IIIa antagonists [366,325]. Thus, the monitoring of antiplatelet therapy is becoming increasingly important and many instruments have been, or will be, utilized as point-of-care instruments for monitoring antiplatelet therapy or for the assessment of bleeding risk [326].

A single dose of 160 mg completely abolishes platelet TXA<sub>2</sub> production [324] and the same effect can be progressively achieved with the chronic administration of daily doses of 30 to 50 mg [29]; however, variable platelet responses to aspirin have been described. Indeed, based on measurements of platelet aggregation in response to arachidonate and ADP, 5 and 24% of patients, respectively with stable cardiovascular disease who were receiving 325 mg aspirin once daily were defined as being 'resistant' and 'semi-responders' [327]. The aspirin-resistant group had an increased risk of death, MI, or cerebrovascular accident during almost 2 years' follow-up. Another study using different techniques to measure platelet aggregation, showed that 57% of a group of 88 patients with documented heart failure who had been treated with aspirin 325 mg/day for over a month, showed 'aspirin nonresponsiveness' [328].

The ADP receptor blocker clopidogrel reduces the incidence of recurrent ischemic events in patients with ACS [220] and after coronary stenting [222]. Platelet inhibition with clopidogrel is evident within two hours of an initial dose and the maximal effect with an initial dose has been noted to occur at 400 mg (40% platelet inhibition). In healthy volunteers ADP-induced platelet aggregation was inhibited within 2 days (platelet inhibition by ~30%), and reached a maximal inhibition (60% platelet inhibition) within 4 to 7 days of continued dosing (50–100 mg/day) of clopidogrel [156]. However, clopidogrel 'nonresponsiveness' has been reported to be present in as few as 5% to as many as 56% of patients who are undergoing coronary stenting. Previous studies [329–331] labeled patients as nonresponders based on the arbitrary definitions of the change in ADP-induced platelet aggregation before and after the start of clopidogrel therapy. Indeed, a significant number of cardiovascular events continue to occur [23,222]. In a more recent study, the antiplatelet effect of clopidogrel was studied prospectively in 60 consecutive patients who underwent PCI with stenting for acute MI to determine whether variability in response to clopidogrel affects clinical outcomes. This study demonstrated that up to 25% of patients undergoing primary PCI with

stenting were resistant to clopidogrel (mean ADP-induced platelet aggregation on day 6 of treatment: 103 ± 8% of baseline) and, therefore, might be at an increased risk for recurrent cardiovascular events [332]. Otherwise, from a secondary post-hoc analysis it has been demonstrated that pretreatment platelet activity and clinical characteristics were not associated with responsiveness to clopidogrel [333]. In this study, platelet function before and after clopidogrel therapy was analyzed in all 544 individuals by conventional aggregometry. Hypo- (4.8%) and hyper-responders (4.3%) to clopidogrel, as determined by change in ADP-induced platelet aggregation, did not significantly differ in clinical characteristics from those whose responses were within the standard range. Moreover, platelet activity before the administration of clopidogrel, which was defined by baseline platelet aggregation response to ADP, did not appear to be associated with the response to clopidogrel.

Measurement of platelet function by the rapid platelet function assay (Ultegra) has been found to predict therapeutic response to GPIIb/IIIa antagonists in patients with PCI [334]. Moreover, there have been several examples of the application of the FDA-approved platelet function analyzer 100 (PFA-100) in therapeutic monitoring [335]. In patients suffering from peripheral arterial occlusive disease poor responders to clopidogrel (detected by the PFA-100) had an increased likelihood of experiencing restenosis after percutaneous angioplasty [336]. Patients with ST-elevated MI had significantly enhanced platelet function when measured under high shear rates with the PFA-100 [337] and standard doses of GPIIb/IIIa antagonists, particularly tirofiban, had a limited impact on high shear-induced platelet formation at physiologic Ca<sup>2+</sup> concentration [322]. Moreover, since GPIIb/IIIa antagonists have been shown to dose dependently inhibit platelet aggregation [322,338] one may speculate that the inefficacy of these antiplatelet drugs could be due to insufficient therapeutic dosing. Indeed, as suggested by the Enhanced Suppression of the Platelet IIB/IIIa Receptor with Integrilin Therapy (ESPRIT) trial [271], both medically treated patients and patients undergoing PCI may benefit from higher dosages of GPIIb/IIIa antagonists.

However, the use and benefit of the monitoring of antiplatelet drugs in clinical settings are currently controversially discussed [324] and further well-designed studies are needed to demonstrate its utility in different populations of patients with vascular disease.

## Potential antidotes

When bleeding occurs under treatment with antiplatelet agents, evaluation of platelet function may also help to guide further decision making. In the acute event, platelet concentrates (PC) or desmopressin (DDAVP) may be helpful.

Concerning the use of PC the pharmacokinetics of GPIIb/IIIa inhibitors is of important practical implication [265]. The plasma levels of unbound abciximab drop very rapidly after administration. The rapid disappearance of free abciximab in plasma is an important consideration for reversing the therapeutic effect. In the acute situation, the transfusion of platelets results in an immediate and partial normalization of platelet function, since the amount of unbound plasma abciximab available to inhibit transfused platelets is very small. In contrast to abciximab, the plasma concentration of eptifibatid molecules at peak dosing is very high relative to the number of GPIIb/IIIa molecules. As a result, newly transfused platelets would probably be rapidly inhibited. Tirofiban is intermediate between abciximab and eptifibatid in its peak molecular concentration relative to the number of GPIIb/IIIa molecules. Thus, platelet transfusions are possibly far more effective after abciximab administration than following tirofiban or eptifibatid infusion.

DDAVP has been shown to accelerate normalization of *in vitro* platelet dysfunction induced by GPIIb/IIIa antagonists [238]. Combined use of platelet concentrates and DDAVP has been shown to additively enhance recovery of normal platelet function after infusion of GPIIb/IIIa antagonists [338]. Thus, based on our previous reports [338,339] and based on the potential necessity to administer large numbers of PC [340] in case of bleeding induced by GPIIb/IIIa inhibitors cautious recommendations may be given: DDAVP should be used whenever bleeding is suspected to stem from aspirin or GPIIb/IIIa inhibitors. As long as there are no clinical trials in bleeding patients, the authors cautiously recommend the following course of action:

- Stop the GPIIb/IIIa infusion
- Obtain a platelet count and monitor activated partial thromboplastin (aPTT) or anti-FXa activity levels when anticoagulants are used concomitantly
- If possible, measure the degree of platelet inhibition with a rapid bedside test

- Administer a DDAVP infusion
- Transfuse platelet concentrates in case of major or life-threatening bleeding or urgent need for normalization of platelet function in case of surgery.

## Expert opinion &amp; outlook

An ideal antiplatelet agent should specifically block thrombogenic platelet-dependent mechanisms in vascular diseases without interfering with normal platelet functions that are required in hemostasis and wound healing. Additionally, these agents should be free of any major adverse events. Although, several antiplatelet strategies have already been developed or are under preclinical or clinical investigation (Table 1) none of the available antiplatelet drugs meet all of these criteria.

Aspirin has been, and will be, the standard reference compound for long-term oral treatment of platelet hyper-reactivity, most notably in the secondary prevention of cardiovascular diseases [39]. However, aspirin is neither selective for platelets nor a potent antiplatelet compound. As platelet activation occurs via several pathways that do not rely on amplification by released TXA<sub>2</sub>, adding a second antiplatelet drug to aspirin might have additional benefits in some clinical circumstances, but more research into this strategy is required.

The combination of modified-released dipyridamole and low-dose aspirin therapy has been approved by the FDA but recommendations for this class of antiplatelet drugs are controversial [162–165,202]. The reduction in non-fatal stroke was derived merely from one large trial [159], but this result was not supported by the findings for nonfatal stroke in other studies or by the overall findings for nonfatal MI or vascular death [39]. Although dipyridamoles are considered to have some benefit in cerebrovascular disease they have not been proven as an effective agent in cardiovascular disease.

Thienopyridines such as clopidogrel have been shown to be beneficial in the treatment of vascular disease. Moreover, thienopyridines are the therapeutic alternative in aspirin tolerance or resistance. In combination with low-dose aspirin, clopidogrel has been shown to be more efficacious than aspirin alone, especially in patients with ACS [222,224,234] and several studies are currently planned or ongoing (Table 6). Newer ADP receptor antagonists will probably become part of clinical practice in the next few years.

Currently, the intravenous GPIIb/IIIa antagonists are the most potent inhibitors of platelet aggregation. Their use is restricted to patients

undergoing PCI and to patients with ACS before revascularization but the role of GPIIb/IIIa antagonists in the noninterventive management of ACS is more controversial [266]. Oral GPIIb/IIIa antagonists have failed to be beneficial in the treatment of vascular disease and it seems unlikely that there will be any further development of these drugs.

To summarize, there is a better understanding of the molecular events regulating thrombogenesis and ongoing investigations are exploring the value of novel antiplatelet agents in various preclinical or clinical trials. Future developments might probably include combined-mode agents targeting one or more steps in the thrombotic process to optimize the efficacy and safety of antiplatelet therapy.

## Highlights

- **Aspirin:** multiple, randomized, controlled clinical trials have shown a clinically significant decrease in cardiovascular morbidity and mortality in patients at risk of recurrent atherothrombotic events. Although the beneficial effect of aspirin in secondary prevention of ischemic events is well established, the role of primary prevention is less clear, particularly in women.
- **Dipyridamole:** the combination of modified-released dipyridamole and low-dose aspirin therapy has been approved by the US Food and Drug Administration; however, recommendations for this class of antiplatelet drugs are controversial.
- **Thienopyridines:** clopidogrel has been shown to be more efficacious than aspirin alone especially in patients with acute coronary syndrome and several studies are currently planned or ongoing. Clopidogrel should not be used when aspirin causes gastrointestinal (GI) intolerance, because aspirin plus esomeprazole was definitely superior to clopidogrel for the prevention of recurrent GI bleeding among patients with a history of aspirin-induced ulcer bleeding.
- **GP IIb/IIIa antagonists:** a large number of trials have been carried out with GPIIb/IIIa antagonists and several studies have shown the benefit of GP IIb/IIIa inhibitors in patients undergoing percutaneous coronary intervention (PCI) and in patients with acute coronary syndrome (ACS) before revascularisation. In contrast, the oral GPIIb/IIIa antagonists have failed to be beneficial in the treatment of vascular disease and it seems unlikely that there will be any further development of these drugs.
- **Monitoring of platelet function:** with the increasing number of anti-platelet drugs, the monitoring of antiplatelet therapy is becoming increasingly important and many devices have been or will be studied as point-of-care instruments for monitoring antiplatelet therapy or assessing bleeding risk
- **Potential antidotes:** when bleeding occurs under treatment with antiplatelet agents evaluation of platelet function may help to guide further decision making. Combined use of platelet concentrates and desmopressin has been shown to additively enhance recovery of normal platelet function after infusion of GPIIb/IIIa antagonists, thus, in the acute event or desmopressin and/or platelet concentrates may be helpful.

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