

# Aspirin and Clopidogrel: A Sweeping Combination in Cardiology

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**Abstract:** Platelets play a pivotal role in the pathogenesis of atherothrombosis, believed to be integrally involved in both the development and progression of atherosclerotic heart disease, as well as in its acute thrombotic complications. Antiplatelet therapy constitutes the cornerstone in the management of patients with acute coronary syndromes and generally high-risk patients with atherothrombosis. Until recently, long-term antiplatelet therapy for the treatment and prevention of the complications of atherothrombotic disease was traditionally limited to aspirin. The availability of the thienopyridines, in particular clopidogrel, represents an important addition to the physician's armamentarium. Clopidogrel is currently one of the most widely prescribed drugs for the treatment of symptomatic coronary artery disease. Aspirin and clopidogrel interfere with platelet activation in complementary, but separate pathways. Aspirin irreversibly inhibits cyclooxygenase, thus preventing the production of thromboxane A<sub>2</sub>, which is a prothrombotic and vasoconstrictive substance. Clopidogrel, a newer thienopyridine which has largely supplanted ticlopidine due to a more favorable safety profile, irreversibly prevents platelet activation by blocking one of the three known adenosine 5'-diphosphate (ADP) receptors (the P2Y<sub>12</sub> receptor) on the platelet surface, thus interfering with platelet activation, degranulation and aggregation. Both these antiplatelet agents have a potent protective effect against adverse vascular events, but the combination of these two agents has an even stronger antiplatelet effect translating into superior antithrombotic protection in coronary, cerebral or peripheral arterial disease, without an inordinate increase in bleeding complications.

A number of seminal clinical trials have demonstrated and confirmed the incremental benefit and efficacy of the combination of clopidogrel and aspirin therapy above and beyond that of aspirin alone, with multiple other important large-scale clinical trials currently ongoing. Newer data are being accumulated from studies where indications for the use of clopidogrel and aspirin continue to expand into other patient groups, rendering this dual antiplatelet drug therapy a sweeping combination in Cardiology. However, important issues remain to be further and more thoroughly explored about the benefit of this antiplatelet drug combination in these other patient groups, such as in patients with *heart failure*, where preliminary data indicate a favorable effect on thrombotic vascular events, in patients with *atrial fibrillation*, where there is hope that this combination may replace or be an alternative treatment modality to coumadin in certain subpopulations, in patients undergoing demanding *catheter ablation* procedures, where data point to a protective effect from thromboembolic events. Another pertaining issue to be further investigated is the occurrence of *drug-resistance* observed in some patients for both these antithrombotic agents. This article is a comprehensive review of all these data and the landmark trials on the two antiplatelet agents, the issues involved and the current recommendations for their use in patients with atherosclerotic heart disease and other cardiovascular disorders and procedures.

**Key Words:** Aspirin, clopidogrel, antithrombotic drugs, ischemia, coronary artery disease, coronary angioplasty, coronary stenting, coronary thrombosis.

## INTRODUCTION

Atherothrombotic coronary artery disease is the single most common cause of death worldwide and a growing public health problem. Platelets play a pivotal role in the pathogenesis of atherothrombosis, believed to be integrally involved in both the development and progression of atherosclerotic heart disease, as well as in its acute thrombotic complications. Intracoronary thrombi produced during atherosclerotic plaque rupture spontaneously or mechanically during percutaneous coronary interventions (PCI) result from activation and aggregation of platelets. Platelets are therefore commonly targeted by antiplatelet drugs as part of strategies of primary and secondary prevention of atherothrombosis.

Antiplatelet therapy constitutes the cornerstone in the management of patients with acute coronary syndromes and generally high-risk patients with atherothrombosis.

Until recently, long-term antiplatelet therapy for the treatment and prevention of the complications of atherothrombotic disease was traditionally limited to aspirin. Aspirin reduces the risk of serious vascular events, such as myocardial infarction, stroke or cardiovascular death, by ~20% in a wide range of high-risk patients and remains the first-line antiplatelet drug because of its relative safety, low cost and cost-effectiveness. Although a remarkably cost-effective therapy, in placebo-controlled clinical trials ~75% of patients at risk continue to experience thrombotic events despite chronic aspirin therapy. The availability of the thienopyridines, in particular clopidogrel, represents an important addition to the physician's armamentarium. Clopidogrel is currently one of the most widely prescribed

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drugs for the treatment of symptomatic coronary artery disease. Compared with aspirin alone, clopidogrel reduces the risk of serious vascular events by ~10% and the combination of aspirin and clopidogrel reduces the risk by ~20% in patients with non-ST-segment elevation acute coronary syndrome. Clopidogrel has a similar safety profile to aspirin, albeit a considerably higher cost. Aspirin and clopidogrel interfere with platelet activation in complementary, but separate pathways (Fig. 1). Both these antiplatelet agents have a potent protective effect against adverse vascular events, but the combination of these two agents has an even stronger antiplatelet effect translating into superior anti-thrombotic protection in coronary, cerebral or peripheral arterial disease, without an inordinate increase in bleeding complications.

A number of seminal clinical trials have demonstrated and confirmed the incremental benefit and efficacy of the combination of clopidogrel and aspirin therapy above and beyond that of aspirin alone, with multiple other important large-scale clinical trials currently ongoing. The exact mechanism of this benefit is still being elucidated but is clearly related to the inhibition of the many consequences of platelet activation--vascular inflammation, endothelial dysfunction, and localized angiogenesis/mitogenesis--and not just aggregation.

Newer data are being accumulated from studies where indications for the use of clopidogrel and aspirin continue to expand into other patient groups, rendering this dual antiplatelet drug therapy a sweeping combination in Cardiology. However, important issues remain to be further and more thoroughly explored about the benefit of this antiplatelet drug combination in these other patient groups.

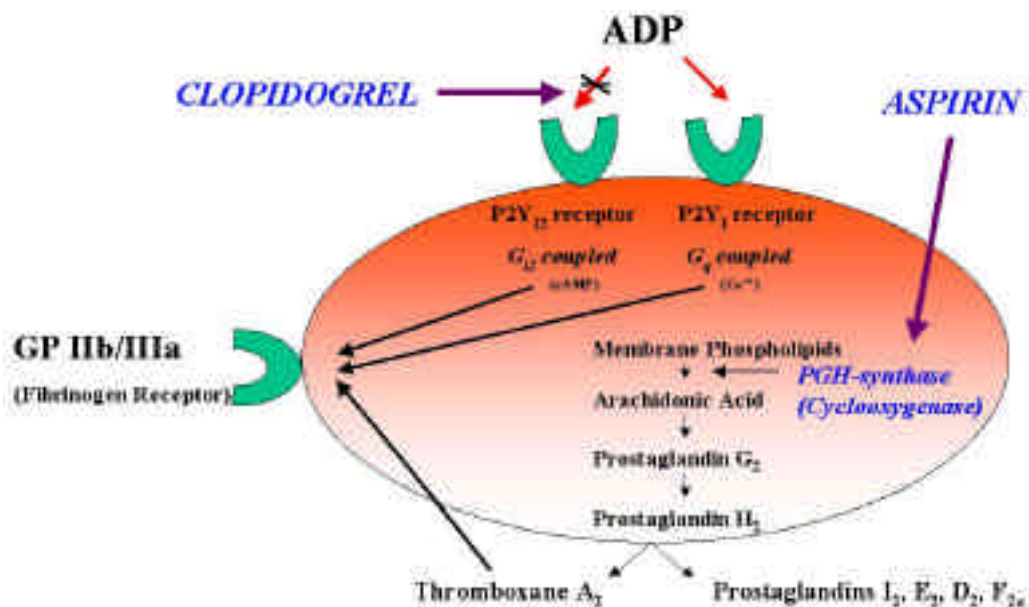
This article is a comprehensive review of all these data and the landmark trials on the two antiplatelet agents, the issues involved and the current recommendations for their use in patients with atherosclerotic heart disease and other cardiovascular disorders and procedures.

## ASPIRIN

Acetyl-salicylic acid (aspirin) (Fig. 2) has a commercial life of approximately 100 years with 35,000 kg of the drug consumed daily in the United States and 60,000 kg in the United Kingdom [1] mainly due to its antipyretic effects. Its anti-platelet action was recognized for the first time in 1953 when L. Craven [2] published his observation about the bleeding tendency of patients taking aspirin. Subsequently, in 1971 Smith and Willis [3] began to unfold the underlying mechanism by demonstrating that aspirin inhibited platelet prostaglandin synthesis. Since then numerous investigators have built their career working on the anti-platelet effect of this famous drug. Moreover, data from several clinical trials have firmly established its important role in secondary prevention, and possibly in primary prevention in selected populations of cardiovascular disease.

### Aspirin Mechanisms of Action

Aspirin performs its anti-platelet action by selectively inactivating the enzyme platelet prostaglandin (PG) H-synthase which is responsible for the formation of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the precursor of thromboxane (TX) A<sub>2</sub> (Fig. 1). The latter, one of the end-products of the arachidonic acid cascade, is released in response to various platelet agonists (platelet activating factor, thrombin, collagen, adenosine diphosphate) and induces irreversible



**Fig. (1).** Sites of action of aspirin and clopidogrel. In the final pathway of platelet aggregation fibrinogen binds on activated glycoprotein (Gp) IIb/IIIa receptors on adjacent platelets. Aspirin and clopidogrel interfere with steps leading to activation of Gp IIb/IIIa receptors. Aspirin inhibits platelets by irreversibly acetylating cyclooxygenase 1 and thereby inhibiting production of thromboxane A<sub>2</sub> which is a potent mediator of platelet aggregation. Clopidogrel inhibits ADP stimulation of the platelet P2Y<sub>12</sub> receptor, thereby inhibiting platelet activation, aggregation, and Gp IIb/IIIa receptor activation.



Fig. (2). The chemical structure of aspirin.

platelet aggregation. PGH-synthase has two distinct catalytic activities: a cyclooxygenase-1 (COX-1) that is involved in the formation of prostaglandin  $G_2$  ( $PGG_2$ ) and a hydroperoxidase responsible for a net two-electron reduction of the  $PGG_2$  molecule resulting in the formation of  $PGH_2$ . Aspirin by irreversibly acetylating the hydroxyl group of a single serine residue at position 529 (Ser<sup>529</sup>) of PGH-synthase does not affect its hydroperoxidase activity but it does inhibit permanently its COX-1 effect [4]. Of note, another isoform of PGH-synthase, COX-2, not routinely present in most mammalian cells but rapidly inducible by inflammatory stimuli and growth factors, has been identified [5]. Aspirin inhibits COX-2 too but at higher concentrations than those required to inhibit COX-1 and this may at least partially explain the different dose requirements for the anti-inflammatory versus the anti-platelet effect of the drug [6].

The COX inhibition effect of aspirin does affect other vasoprotective end-products of the arachidonic acid cascade, like prostaglandin  $I_2$  ( $PGI_2$ ), this way promoting thrombosis and vasoconstriction. The available data, however, suggest that these effects have little clinical relevance and that the antithrombotic effects of  $TXA_2$  inhibition by aspirin predominates [7]. Other mechanisms have also been proposed to fully explain the cardioprotective effects of aspirin. Aspirin seems to protect both LDL cholesterol [8] and endothelial cells [9] from oxidation and to reduce the inflammatory response [10] counteracting this way the main mechanisms responsible for the initiation and progression of atherosclerosis. Furthermore, experimental evidence suggests a direct vasoprotective effect of aspirin, whereby aspirin protects endothelial cells from oxidative stress. Such an effect may include changes in the expression of lectin-like receptor LOX-1 and matrix metalloproteinase-1 (MMP-1) [11]. These data suggest that aspirin may protect endothelial cells from detrimental effects of oxidized LDL such as increased generation of superoxide and stimulation of LOX-1 and MMP-1 expression.

#### Aspirin Pharmacokinetics / Pharmacodynamics

Aspirin is rapidly absorbed in the upper gastrointestinal (GI) tract and results in a measurable inhibition of platelet function within 60 minutes [7,12]. These effects occur even before acetylsalicylic acid is detectable in the peripheral blood, owing to the exposure of platelets to aspirin in the portal circulation [13]. The plasma half-life of aspirin is only 20 minutes; however, because platelets cannot generate new COX, the effects of aspirin last for the duration of the life of the platelet (~10 days). After a single dose of aspirin, platelet COX activity recovers by ~10% per day as a function of platelet turnover [14]. Although it may take 10 days for the total platelet population to be renewed, and thus restore

normal COX activity, it has been shown that if as little as 20% of platelets have normal COX activity, hemostasis may be normal [15,16]. Aspirin is an effective antithrombotic agent in a wide range of daily doses (30-1500 mg/d). Daily aspirin doses of 75-150 mg seem to be as effective as higher doses for long-term treatments but in acute settings an initial loading dose of at least 150 mg of aspirin may be required [17].

#### Aspirin in Acute Myocardial Infarction

The Second International Study of Infarct Survival (ISIS-2) [18], a cornerstone trial, established the role of aspirin as an integral part of the therapeutic strategy in acute myocardial infarction (MI). In this trial, 17187 patients presenting within 24 hours of the onset of a suspected acute MI were randomized to intravenous streptokinase, aspirin (162.5 mg), their combination or placebo. Aspirin alone resulted in a 21% reduction in 5-week vascular mortality compared to control, which remained significant at 15-month follow-up. Aspirin also reduced the incidence of nonfatal reinfarctions (1% aspirin vs. 2% control) and nonfatal strokes (0.3% aspirin vs. 0.6% control) without significant bleeding complications. This mortality benefit was maintained after 10 years of follow-up [19].

Another important finding of ISIS-2 was the additive beneficial effect that aspirin has to the effect of streptokinase when the two drugs were combined (42% reduction in vascular death). Aspirin remains thereafter an important adjunct to thrombolysis although in comparison with heparin it appears to be associated with lower early patency rate of the infarct-related artery [20]. Nevertheless, a meta-analysis of 32 trials demonstrated that aspirin as an adjunct to thrombolysis significantly decreased reocclusion rates (11% with aspirin vs. 25% without aspirin) and recurrent ischemic events (25% with aspirin vs. 41% without aspirin) [21]. The addition of heparin to aspirin has not shown an overall significant clinical benefit [22,23], while recent data suggest that the combination of aspirin with warfarin or coumadin may be more promising [24,25].

#### Aspirin Post-Myocardial Infarction

Aspirin is a simple, highly cost-effective secondary prevention intervention in patients with a history of myocardial infarction (MI). The Antiplatelet Trialists' Collaboration [26] conclusively demonstrated its value by performing a meta-analysis of 174 randomized trials of antiplatelet therapy for cardiovascular disease. Aspirin reduced the rate of recurrent ischemia by 10-14% in the approximately 20,000 patients with acute MI who were included in this meta-analysis. A similar beneficial effect was shown in the ISIS-2 [18] where aspirin was given for 1 month after acute MI. Nevertheless, aspirin remains relatively underused in the post-MI patients especially in the elderly [27].

#### Aspirin in Unstable Angina and Non-ST Elevation Myocardial Infarction

Several studies have provided compelling evidence that aspirin significantly reduces the incidence of death, recurrent

MI, recurrent angina or progression to severe angina necessitating cardiac catheterization and nonfatal stroke in this category of patients [28-31]. In these studies, aspirin has been given in different doses (75-1300 mg) and at various intervals after the patient's presentation (< 24 hours to <8 days). Nevertheless, the beneficial effect has been consistent (5% average absolute risk reduction in non-fatal stroke, MI or vascular death) [26]. In comparison to heparin [32-34], most but not all [28] studies have shown an inferiority of aspirin while their combination seems to be offering additional benefit of borderline significance [35] possibly at the expense of an increase in bleeding complications [32]. A recent study among 1,236 patients hospitalized for acute coronary syndrome indicated that aspirin withdrawal in coronary patients poses a real risk for the occurrence of a new coronary event, many cases involving late uncoated-stent thrombosis [36].

### **Aspirin in Stable Angina**

The evidence about the benefits of aspirin in patients with stable angina comes from the results of the Swedish Angina Pectoris Aspirin Trial [37], a prospective study of 2,035 patients with stable angina which revealed that 75 mg of aspirin added to sotalol produced a 34% decrease in primary outcome events of myocardial infarction and sudden death (95% confidence interval of 24% to 49%,  $p = 0.003$ ) and a 32% reduction in secondary vascular events. To further support the use of aspirin in this setting in the Physician's Health Study, patients who had chronic stable angina and received aspirin had an 87% reduction in the risk of MI compared with those who were taking only placebo [38].

### **Aspirin in Cerebrovascular Disease**

Two large randomized trials [39,40], which enrolled >40,000 patients within 48 hours of the presentation of an acute ischemic stroke, demonstrated a significant decrease in the risk of recurrent stroke and the combined incidence of death or nonfatal stroke. The addition of heparin did not offer any further benefit while increased the risk of intracranial bleed [39]. After the acute event, aspirin remains the first-option drug for the prevention of a recurrent ischemic attack as has been shown in multiple clinical trials [41-43]. In a recent meta-analysis [17], among 18,270 patients in 21 trials, allocation to a mean duration of 29 months of aspirin (and other antiplatelet agents too) resulted in 36 fewer major vascular events per 1000 patients, a benefit mainly reflecting a highly significant reduction in non fatal strokes (25 fewer per 1000 patients). Although some controversy still exists, treatment with aspirin at medium doses (75 to 325 mg/d) is the most widely accepted therapeutic regimen. Recent reviews of randomized trials of antiplatelet therapy [17,44] did not find any significant difference regarding protective effects between high aspirin doses (500 to 1500 mg/d) and medium to low doses (30 to 325 mg/d). However, compared with medium doses, high doses of aspirin showed an increased risk of adverse gastrointestinal effects, while no significant differences were observed between medium (75 to 325 mg/d) and low doses (<75 mg/d) [45]. There is no evidence that reducing the dose of aspirin would significantly lower its hemorrhagic risk [46].

### **Aspirin in Primary Prevention of Cardiovascular or Cerebrovascular Disease**

In contrast to the existing strong evidence about the protective role of aspirin against a second ischemic event, primary prevention data have been less convincing. In low risk male populations, aspirin has been shown to decrease the risk of nonfatal MI without affecting cardiovascular mortality. In addition an increase of nonfatal bleeding and of stroke, primarily of the hemorrhagic type, has been noted.

In the Physician's Health Study [47] where 22,071 male subjects between the ages of 40 and 84 years were randomized to aspirin (325 mg every other day) or placebo, a 44% reduction in the incidence of nonfatal MI was found in the 5 years of follow up. However, total cardiovascular mortality was not affected by aspirin, while a significant increase in gastrointestinal bleeding requiring transfusion and a non-significant increase in hemorrhagic stroke were reported. Another major primary prevention trial was the British Physician's Study [48], which enrolled 5139 subjects with a mean follow up of 6 years. This trial did not show any differences in cardiovascular mortality or in incidence of MI and even though the incidence of transient ischemic attack (TIA) was lower in the aspirin arm, this beneficial effect was outweighed by a significant increase in the incidence of disabling strokes (19.1% vs. 7.4%,  $P < 0.05$ ). Finally, in another small study of asymptomatic patients with carotid bruits, aspirin failed to prevent subsequent cerebrovascular events [49]. No randomized data exist for the female population and the only available evidence comes from the Nurses Health Study [50], where the intake of up to 6 aspirins per week did not show to alter the risk of a cardiovascular event or stroke.

In summary, aspirin prophylaxis in low risk population does not seem to have any significant vascular protective effect and its use should be possibly restricted only to high risk subgroups. Such high-risk subgroups would be diabetic patients and/or patients with manifest vascular disease, particularly those who have experienced a myocardial infarction or other vascular event [51,52].

### **Adverse Effects of Aspirin**

The inhibition of prostaglandin synthesis by aspirin, beyond its favorable anti-inflammatory and anti-platelet effects, is also responsible for the loss of protective prostaglandin function on gastric mucosa [53] and of prostaglandin vasodilatory effect on renal circulation [54]. The resultant gastric toxicity varies from minor dyspepsia with nausea, vomiting and heartburn to peptic ulcers complicated by major bleeding in <1% of the cases. This gastric toxicity is clearly dose-related [53], but is not eliminated in the low doses [55] leading occasionally to discontinuation of therapy [28,56].

On the other hand, aspirin is associated, to a lesser degree, with an increased risk of renal failure compared to the nonsteroidal anti-inflammatory drugs [31]. However, at high doses it has been shown to decrease sodium renal excretion in patients with heart failure and to attenuate the vasodilatory effect of angiotensin converting enzyme (ACE)

inhibitors in patients with heart failure or hypertension [57]. Also, with regard to the bleeding effect of aspirin, although it attenuates the risk of a thrombotic event, on the other hand it significantly increases the risk of a hemorrhagic stroke [58]. Finally, the risk of developing bronchoconstriction, rhinitis or urticaria in a few sensitive to aspirin individuals should not be overlooked [59].

### Aspirin Resistance

Despite the well documented benefit of aspirin in the secondary prevention as well as its possible beneficial effect in selected populations for primary prevention of vascular disease, a large segment of the population at risk does not benefit from aspirin. This has been partially attributed to the so called aspirin resistance. As aspirin resistance, a poorly defined term, is considered the failure of aspirin to prevent the recurrence of a vascular event in 10-20% of the patients with a history of a vascular thrombosis who are taking the drug for secondary prophylaxis [60].

Three explanations of this phenomenon have been postulated: the ability of platelets to become activated by pathways that are not blocked by aspirin, the requirement of higher than the conventional doses (75-325 mg) to achieve optimal anti-platelet effect in some patients and the ability of others to generate thromboxane A<sub>2</sub> (TXA<sub>2</sub>) despite the administration of therapeutic doses of aspirin [61]. A common genetic polymorphism of glycoprotein IIIa, found in 20-30% of Caucasians and associated with shortened bleeding time and enhanced thrombin formation, may also be responsible at least in particular patients [62].

Since aspirin resistance is defined on clinical grounds it has been difficult to show an association with laboratory parameters of platelet dysfunction. Nevertheless, Eikelboom *et al.* [63] recently suggested that urinary levels of 11-dehydro thromboxane B<sub>2</sub>, a major metabolite of TXA<sub>2</sub> might be a useful index of aspirin resistance associated more importantly with increased risk of MI and cardiovascular death.

The clinical importance of aspirin resistance is further underlined by the results of major clinical trials including the recently published Clopidogrel in Unstable angina to Prevent Recurrent events (CURE) trial [64], which demonstrated the superiority of the platelet ADP-receptor clopidogrel plus aspirin over aspirin alone in the prevention of recurrent ischemic events and death in high-risk patients [65-67].

### CLOPIDOGREL

Clopidogrel (Fig. 3) is a member of the thienopyridine family with structural differences in comparison to ticlopidine, which albeit minor, are responsible for its enhanced pharmacological activity combined with a favorable safety profile.

### Mechanism of Action of Clopidogrel

Clopidogrel's antiplatelet effect is mediated by an active hepatic metabolite [68], which irreversibly inhibits ADP membrane receptors located on platelet surface. Two G protein-coupled membrane receptors, P2Y<sub>1</sub> and P2Y<sub>12</sub>, are

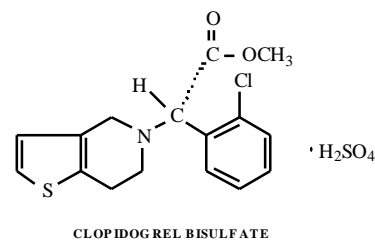


Fig. (3). The chemical structure of clopidogrel.

implicated in ADP-induced platelet aggregation [69]. The P2Y<sub>1</sub> receptor acting through a G<sub>q</sub> second-messenger stimulates calcium mobilization resulting in a platelet shape change and initiation of aggregation [70-72], while the P2Y<sub>12</sub>, a G<sub>12</sub> coupled receptor, is responsible for amplification of platelet aggregation and thrombus stabilization [73,74]. The latter receptor is irreversibly antagonized by clopidogrel, thereby inhibiting ADP-mediated induction of the platelet glycoprotein (GP) II<sub>b</sub>III<sub>a</sub> receptor, the final common pathway of platelet aggregation (Fig. 1) [73,75].

Apart from clopidogrel's blocking effect on the P2Y<sub>12</sub> receptor, several additional mechanisms of action have also been proposed. Clopidogrel attenuates platelet aggregation induced by thrombin [76], a rather indirect effect attributed to inhibition of P2Y<sub>12</sub>-induced amplification of thrombin's agonist effect [77,78]. *In vitro* studies have shown that clopidogrel reduces myointimal thickening after endothelial injury [79]. Clopidogrel also diminishes platelet expression of CD40 ligand and P-selectin [80,81], which have a documented role in atherosclerosis progression [82] and plaque destabilization [83] and exerts an anti-inflammatory effect by reducing C-reactive protein (CRP) levels [80,84,85]. However, contradictory evidence exists regarding clopidogrel's effect on the soluble forms of CD40 ligand and P-selectin [76,80,86]. It should also be noted that although thienopyridines have been reported to stimulate fibrinolytic activity, independent of their antiplatelet effects [87], no significant induction of fibrinolysis has been demonstrated in humans following an oral loading dose of 300 mg in healthy volunteers [88].

Furthermore, there is evidence that the P2Y<sub>12</sub> receptors are expressed in vascular smooth muscle cells, and ADP acting on P2Y<sub>12</sub> receptors not only is important for platelet activation but also stimulates vasoconstriction. Thus, drugs, like clopidogrel, with antagonistic effects on P2Y<sub>12</sub> receptors, affecting both platelets and vascular smooth muscle cells, could be of double therapeutic benefit in their prevention of both thrombosis and vasospasm [89].

### Pharmacokinetics of Clopidogrel

Following oral ingestion approximately 50% of clopidogrel is absorbed from the gastrointestinal tract. The bioavailability of clopidogrel is unaffected by food. Clopidogrel is rapidly hydrolyzed *in vivo* by esterases to an inactive carboxylic acid metabolite, SR26334. The parental drug is undetectable in the plasma 2 hours after oral administration [90]. The serum half-life of SR26334 is 8 hours. Clopidogrel is also enzymatically converted by cytochrome P450 isoform

3A4 (CYP3A4) to an active thiol derivative which forms a disulfide bond with the ADP receptor [91]. Due to the irreversible inhibition of the covalently modified target receptor, the drug's biologic half-life is quite long and is dependent on platelets' turnover rate. Radiolabeling studies in healthy volunteers have demonstrated that approximately 50% and 46% of <sup>14</sup>C-labelled clopidogrel is eliminated in the urine and faeces, respectively, within 5 days of oral administration. However, a small percentage, approximately 2% of the radiolabel, is excreted very slowly, with an elimination half-life of 11.5 to 18 days, seemingly representing the platelet bound radiolabeled drug [92]. Dosage should not be adjusted in elderly subjects or in patients with renal or mild hepatic impairment [93]. However, clopidogrel administration should be avoided in patients with severe hepatic impairment as well as in those exhibiting bleeding diathesis. The drug should be used during pregnancy (and breast feeding) only if clearly needed due to the lack of data regarding its safety in pregnant or lactating women.

After administration of single oral doses in healthy volunteers, clopidogrel inhibits ADP-induced platelet aggregation in a dose-dependent manner, with a maximum inhibition of 40% to 50% after a single 400 mg oral dose. The inhibition of platelet aggregation is detectable 2 hours after oral dosing of 400 mg and remains relatively stable up to 48 hours [94]. With repeated daily dosing of 50 to 100 mg of clopidogrel in healthy volunteers, ADP-induced platelet aggregation is inhibited from the second day of treatment (25 to 30% inhibition) and a steady state (50 to 60% inhibition) is achieved after 4 to 7 days. The maximal platelet inhibition induced by clopidogrel is comparable to that achieved with 500 mg daily dosing of ticlopidine. However, clopidogrel displays a more rapid onset of antiplatelet effect in comparison to ticlopidine.

Studies in healthy subjects have shown that a regimen comprising a 300 mg loading dose followed by 75 mg daily dose, provides a rapid onset of action combined with a sustained steady-state level of platelet inhibition [93]. However, a loading dose of 600 mg results in a more rapid achievement of steady-state levels as well as in a more intense and rapid inhibition of platelet activation and aggregation [95,96]. A recent study has shown that a 600 mg loading dose given to patients without ongoing clopidogrel treatment inhibits platelet aggregation in a similar degree to that achieved by chronic therapy with 75 mg daily [97]. Furthermore, the administration of a 600 mg loading dose to patients already on chronic clopidogrel treatment with 75 mg/d results in further platelet inhibition [97]. Following drug discontinuation, platelet function recovers in 5 to 7 days.

### Drug Interactions

Clopidogrel is an inactive prodrug which is converted to an active metabolite by cytochrome P450 3A4 (CYP3A4). Taking into consideration that about half of all drugs currently used in clinical practice are metabolized by CYP3A4 [98], clopidogrel is subject to several drug interactions with other CYP3A4 substrates. Co-administration of clopidogrel with CYP3A4 inhibitors, such as erythromycin

or troleandomycin, in healthy volunteers, has been shown to inhibit the activation and consequently the antiplatelet effect of clopidogrel [99]. On the contrary, CYP3A4 inducers such as rifampicin enhance the inhibition of platelet aggregation mediated by clopidogrel [99].

Clopidogrel's interaction with several statins has been extensively studied in the literature. Only statins metabolized by CYP3A4 are prone to interfere with clopidogrel, since the molecular base of this potential interaction is a competitive antagonism of substrates sharing a common metabolic pathway. The CYP3A4 enzyme system is primarily implicated in the metabolism of atorvastatin, simvastatin and lovastatin, contributes to a lesser extent in the metabolism of fluvastatin, while rosuvastatin and pravastatin are eliminated by other metabolic routes [100].

Atorvastatin was the first statin reported to blunt platelet aggregation inhibition by clopidogrel [91,99]. The impact of this drug interaction is rather complex and seems to be dependent on the duration of co-administration. During the loading phase of treatment, atorvastatin administration in doses widely used in clinical practice, has been shown to attenuate clopidogrel's antiplatelet effect *ex vivo* in a dose-dependent manner [99,101]. However, since atorvastatin is a competitive inhibitor of clopidogrel metabolism by CYP3A4, increasing clopidogrel's concentration would be expected to blunt or even overcome this antagonism. This hypothesis was confirmed by Muller *et al.* who demonstrated that the inhibition of platelet aggregation by a high (600 mg) loading dose of clopidogrel was not significantly altered by concomitant treatment with atorvastatin or any statin metabolized by CYP3A4 [102].

Existing evidence supports that the abovementioned drug interaction, even if significant during the loading phase, does not have any adverse effect either at the level of platelet function or on patients' clinical outcome during the maintenance phase of treatment. The attenuation of clopidogrel's antiplatelet effect during atorvastatin co-administration has been demonstrated to diminish after 48 hours (maintenance phase of therapy) [101]. Mitsios *et al.* demonstrated that 10 mg of atorvastatin, a rather low dose to cause significant drug interaction, does not influence the antiplatelet efficacy of clopidogrel following co-administration for 5 weeks in patients with acute coronary syndrome [103]. According to a retrospective analysis of the PRONTO trial in 100 patients undergoing coronary stent implantation, platelet inhibition by clopidogrel was unrelated to concomitant treatment with statins [104]. Furthermore, a post-hoc analysis of the CREDO trial argued against any significant clinical impact of this drug interaction, since co-administration of clopidogrel with statins metabolized by CYP3A4 was not associated with any effect on clinical event rates either after 28 days or 1 year of follow-up [105]. Similarly, the results from the MITRA PLUS registry showed that the clinical outcome of patients with acute coronary syndromes receiving clopidogrel therapy was not influenced by the type of statin administered over a 14-month follow-up period [106]. The impact of statin co-administration on clopidogrel's antiplatelet effect was further clarified by the results of the recently published INTERACTION study. This prospectively designed trial showed that the platelet-related effects of

clopidogrel were nearly identical among patients undergoing coronary stenting who had been treated for 30 days with atorvastatin, other statins or no statins [107].

Despite the existing divergence of opinion, the weight of evidence supports that the interaction of clopidogrel with atorvastatin or statins in general, does not seem to be of any clinical significance. Thus, the existing data are not considered sufficient to recommend avoidance of co-administration of these widely used agents in everyday clinical practice.

### Clinical Results of Clopidogrel Use

The cornerstone of clopidogrel's clinical usefulness is the CAPRIE study, a large randomized, double-blind, phase III clinical trial which enrolled 19,185 patients with three different clinical manifestations of atherosclerotic vascular disease (recent ischemic stroke, recent myocardial infarction, or symptomatic peripheral arterial disease) and directly compared clopidogrel (75 mg daily) to aspirin (325 mg daily) in secondary prevention of ischemic events. The main finding was that the relative risk of ischemic stroke, myocardial infarction or vascular death was reduced by 8.7% ( $p=0.043$ ) among patients treated with clopidogrel, providing evidence that clopidogrel, if not modestly more effective, is at least as effective as medium-dose aspirin [108]. Interestingly, a subgroup analysis of the CAPRIE patient population showed that clopidogrel significantly reduced the risk of myocardial infarction alone, by 19.2% ( $p=0.008$ ), in comparison to aspirin [109]. Post-hoc analyses of the CAPRIE and CURE studies have shown that clopidogrel's treatment benefit over aspirin is amplified in high-risk subgroups which display an elevated risk of recurrence, such as patients with diabetes [110] and those with prior symptomatic atherosclerotic disease [111,112]. Also, CAPRIE showed that treatment with clopidogrel results in a significant decrease in the need for rehospitalization for ischemic events or bleeding compared with aspirin [113].

### Clopidogrel in Peripheral Arterial Occlusive Disease

Peripheral arterial occlusive disease (PAOD) is a clinical manifestation of atherosclerotic disease with an estimated prevalence of symptomatic disease of 2 to 3% in men and 1 to 2% in women older than 60 years [114,115]. Atherosclerosis is a multisystemic process affecting all vascular beds since the vast majority of patients with PAOD have either manifest or occult coronary or cerebrovascular disease. Antiplatelet agents apart from exerting a favorable effect on the natural course of patients with PAOD largely by preventing thrombotic occlusion of stenotic vessels, they also reduce the occurrence of other vascular events such as ischemic stroke, myocardial infarction and vascular death. The CAPRIE study established the role of clopidogrel in the management of patients with manifestations of atherosclerotic peripheral arterial disease, such as intermittent claudication, previous leg amputation following a history of claudication, history of reconstructive surgery, or preceding angioplasty with no persisting complications following the intervention. The analysis for the subgroup of patients with peripheral arterial disease showed that clopidogrel significantly reduced the average event rate per year compared with aspirin

(3.71% vs 4.86%,  $p=0.0028$ ) which corresponds to a 23.8% relative risk reduction of the primary combined endpoint [108]. Taking also into consideration that the overall safety profile of clopidogrel is at least as good as that of medium dose aspirin, it is obvious that the cost to benefit ratio of clopidogrel favors its use in patients with PAOD. The guidelines of the seventh American College of Chest Physicians (ACCP) conference on antithrombotic therapy for PAOD recommend clopidogrel treatment in comparison to no antiplatelet therapy (Grade 1C), but placing a relatively high value on avoiding large expenditures to achieve small reductions in vascular events, suggest the use of aspirin instead of clopidogrel (Grade 2A) [116]. However, a recent analysis indicates that clopidogrel is cost-effective in patients with peripheral arterial disease since the benefit derived by clopidogrel treatment in this subgroup of patients is achieved at a cost that is within traditional societal limits [117].

### Clopidogrel in Non-Cardioembolic Ischemic Stroke

In the CAPRIE study, despite clopidogrel's superior efficacy over aspirin in reducing the combined risk of ischemic stroke, myocardial infarction, or vascular death in patients with atherosclerotic vascular disease, no significant treatment benefit has been demonstrated in patients with ischemic stroke. In the subgroup of patients with recent ischemic stroke, long-term clopidogrel administration resulted in a 7.3% relative risk reduction of the primary combined endpoint compared to aspirin, which though did not reach statistical significance [108]. However, it should be noted that CAPRIE was not powered enough to detect a significant treatment effect in each subgroup and thus the "negative" result in the ischemic stroke subgroup might reflect a lack of statistical power and not a lack of treatment effect. Based on the above evidence, the guidelines of the seventh ACCP conference on antithrombotic and thrombolytic therapy for ischemic stroke recommend clopidogrel 75 mg daily as an acceptable option for secondary prevention in patients who have experienced a noncardioembolic stroke or transient ischemic attack (TIA) (Grade 1A) [118]. For patients allergic to aspirin, clopidogrel administration is recommended (Grade 1C+). Furthermore, the writing committee has made a suggestion in favor of clopidogrel therapy compared to aspirin therapy (Grade 2B recommendation), placing a relatively high value on a small non-significant risk reduction in stroke rates and a relatively low value on the high cost of clopidogrel in relation to aspirin [109]. However, according to the European Society of Cardiology (ESC) Expert Consensus document on the use of antiplatelet agents, the additional benefit of clopidogrel over aspirin, if any, is considered of statistical uncertainty and clopidogrel has not been granted a claim of superiority versus aspirin [119]. Regarding the issue of cost-effectiveness, Schleinitz *et al.* reported that the cost to benefit ratio of clopidogrel therapy for the secondary prevention of ischemic events in patients with a recent stroke is considered acceptable [117].

### Clopidogrel in Cardioembolic Ischemic Stroke

Approximately 20% of all ischemic strokes are due to cardiogenic embolism, while a substantial percentage of the

cryptogenic ischemic strokes, which account for 30% of the total ischemic stroke burden, are considered to be of cardiac origin [120]. Atrial fibrillation is the primary cause of cardioembolic ischemic strokes although several other cardiac abnormalities such as recent MI, dilated cardiomyopathy, mitral stenosis, infective endocarditis, atrial myxoma are associated with increased incidence of ischemic stroke. For primary prevention of cardioembolic ischemic stroke among patients with non-valvular atrial fibrillation, clopidogrel can be administered as an alternative to aspirin in low-risk patients with true allergy to aspirin. According to the 7<sup>th</sup> ACCP conference on antithrombotic and thrombolytic therapy, in patients with cryptogenic ischemic stroke and a patent foramen ovale, antiplatelet therapy is recommended over no therapy (Grade 1C), while antiplatelet administration is suggested over anticoagulation (Grade 2A). In patients with mitral valve strands or prolapse, who have a history of TIA or stroke, antiplatelet therapy is recommended (Grade 1C) [118]. In these clinical indications clopidogrel could be administered when aspirin administration is contraindicated, while the efficacy and safety of combined antiplatelet treatment has not been evaluated.

### **Clopidogrel in Patients After Coronary Artery Bypass Grafting**

Antiplatelet therapy is of primary importance in patients subjected to coronary artery bypass grafting (CABG) in order to prevent graft occlusion as well as to reduce the elevated risk for recurrent ischemic events due to the diffuse native coronary artery disease, the greater prevalence of coronary risk factors and the atherosclerotic involvement of multiple vascular beds.

In a subgroup analysis of prior cardiac surgery patients with a subsequent qualifying ischemic event such as myocardial infarction, stroke or limb ischemia, enrolled in the CAPRIE trial, Bhatt *et al.* investigated whether clopidogrel was more effective than aspirin in reducing recurrent ischemic events [121]. Clopidogrel therapy resulted in a significant reduction in the annual event rates for all-cause mortality, vascular death, myocardial infarction, stroke, and re-hospitalization, supporting clopidogrel's superiority over aspirin in secondary prevention of ischemic events in a selected group of patients at somewhat higher risk than the typical cardiac surgery patient. However, since in this trial clopidogrel was not started until after surgery, its reported beneficial effect can not be extrapolated to the early postoperative period. Furthermore, a prospective, randomized, controlled clinical trial demonstrated that clopidogrel treatment, in a 75 mg daily regimen without though a loading dose, does not inhibit platelet aggregation in the first five postoperative days [122].

In consistence with the abovementioned evidence, the seventh ACCP conference evidence based guidelines on prevention of coronary artery bypass graft occlusion recommend the administration of 300 mg clopidogrel, as a loading dose, 6 hours after operation, followed by 75 mg daily, only for patients with coronary artery disease undergoing CABG who are allergic to aspirin (Grade 1C) [123]. According to the recently published ACC/AHA guidelines, aspirin is the drug of choice for prophylaxis

against early saphenous vein graft closure, while clopidogrel, having a more favorable safety profile compared to ticlopidine, can be used as an alternative in the truly aspirin allergic patient instead of ticlopidine [124].

Preoperative clopidogrel administration in combination with aspirin in patients undergoing non-emergent CABG is associated with higher postoperative bleeding, higher need for surgical exploration and increased morbidity compared to those without preoperative exposure within seven days [125,126]. Results from a retrospective study demonstrated that patients that received clopidogrel alone 7 days before CABG were at a higher risk of blood transfusion compared to those on aspirin alone or on neither antiplatelet drug [127]. However, contradictory results have also been reported [128]. The seventh ACCP conference guidelines recommend clopidogrel discontinuation for 5 days prior to the scheduled surgery in patients who have received clopidogrel for acute coronary syndromes and are scheduled for CABG surgery (Grade 2A) [123], while the ACC/AHA Task Force recommend clopidogrel withholding for at least 5 days and preferably for 7 days before planned CABG surgery [124].

### **Clopidogrel in Acute Coronary Syndromes**

To our knowledge, a direct head-to-head comparison of clopidogrel with aspirin or with placebo has never been conducted in patients with acute coronary syndromes. However, based on the equivalent efficacy of aspirin and clopidogrel in secondary prevention of ischemic events [108], clopidogrel administration to hospitalized patients with acute coronary syndromes who are unable to take aspirin because of hypersensitivity or major gastrointestinal intolerance, mainly recent significant bleeding from peptic ulcer or gastritis, is considered as a Class I recommendation [129,130].

### **Safety Profile of Clopidogrel**

Data derived from the CAPRIE study have provided evidence that clopidogrel is at least as safe as medium-dose aspirin [108]. The incidence of early permanent discontinuation of the study drug due to reported adverse events was similar in both treatment groups (11.94% for clopidogrel vs 11.92% for aspirin) [131]. The overall frequency of bleeding disorders was identical among aspirin and clopidogrel-treated patients, although the incidence of gastrointestinal haemorrhage was significantly higher with aspirin than with clopidogrel treatment. Gastrointestinal discomfort (indigestion, nausea and vomiting) occurred in significantly more aspirin-treated patients, while severe rash and diarrhea was significantly more frequent among clopidogrel recipients. It should be noted that despite the well-established association of ticlopidine, the other member of the thienopyridine family, with neutropenia [132], no excess neutropenia was reported among clopidogrel-treated patients in the CAPRIE trial. Furthermore, the frequency of thrombocytopenia was identical in both treatment groups.

Despite the overall favorable safety profile of clopidogrel, physicians should be aware of the possibility of rare adverse effects, such as thrombotic thrombocytopenic

purpura (TTP) [133-138], haemolytic uremic syndrome [136,137,139,140], bone marrow failure [141-143] and acquired haemophilia A [144], occurring within days after the initiation of clopidogrel treatment. Clopidogrel-associated TTP usually occurs within the first two weeks of treatment onset, occasionally relapses and has a high mortality rate unless promptly identified and treated with plasma exchange [145]. Taking into consideration the growing number of stent implantation, as well as the expanding indications of clopidogrel administration, a heightened vigilance for these rather rare adverse effects is warranted.

### Clopidogrel Resistance

The platelet inhibitory response to clopidogrel therapy exhibits a marked inter-individual variability raising the issue of clopidogrel resistance [146-149]. A high variability in therapeutic response to clopidogrel has been documented among healthy volunteers [150], patients with stable coronary artery disease [147,149,150], as well as among ST-elevation myocardial infarction (STEMI) patients subjected to primary percutaneous coronary intervention (PCI) [151]. Clopidogrel resistance has been empirically defined as <10% reduction in platelet aggregation in response to ADP (5  $\mu\text{mol/L}$  or 20  $\mu\text{mol/L}$ ) in comparison to pretreatment values [147,149]. The percentage of non-responders among patients undergoing elective coronary artery stent implantation ranges from 10 to 31% [147,149,150], while 16% of healthy volunteers [10] and 25% of patients with STEMI have been reported to be non-responders [151].

The etiology of the response heterogeneity has not been fully clarified. Several underlying mechanisms have been proposed, such as differences in intestinal clopidogrel absorption [152], genetically determined or drug-induced variability in the metabolic activity of CYP3A4 which converts the inactive clopidogrel to its active metabolite [150], increased pre-treatment platelet reactivity [147,153], polymorphisms of the P2Y<sub>12</sub> gene [154], defects in posttranscriptional or posttranslational regulation of the target receptor, or in post-receptor signal transduction cascades.

A few studies have addressed the pivotal issue of whether clopidogrel resistance is associated with an adverse clinical prognosis. Matetzky *et al.* reported that the lack of response to clopidogrel treatment (300 mg loading dose followed by 75 mg daily for 3 months) among STEMI patients subjected to primary PCI may be a marker of increased risk of recurrent cardiovascular events during a 6-month follow-up period [151]. Muller *et al.* in a study that was not designed to prospectively evaluate the clinical significance of clopidogrel resistance, reported that 2 out of 105 evaluated patients who developed subacute stent thrombosis were clopidogrel non-responders [149].

Despite the sparse data available on the clinical significance of clopidogrel resistance, the identification of non-responders by accurate and reproducible tests and the use of therapeutic manoeuvres to cope with the resistance to clopidogrel treatment might decrease the incidence of subacute stent thrombosis which ranges from 1 to 3% even

among patients receiving combined antiplatelet treatment [155,156]. Based on the above considerations, administration of higher loading and maintenance dosage might overcome the resistance to clopidogrel antiplatelet effect, with particular benefit in patients with high baseline platelet reactivity [157]. Furthermore, pharmacologic manipulation of CYP3A4 activity might decrease the incidence of clopidogrel resistance, since co-administration of clopidogrel with CYP3A4 inducers, such as rifampicin, in clopidogrel non- or low-responders has been reported to significantly enhance platelet inhibition [150].

### ASPIRIN AND CLOPIDOGREL

Aspirin and clopidogrel interfere with platelet activation in complementary, but separate pathways. As detailed above, both these antiplatelet agents have a potent protective effect against adverse vascular events. However, the combination of these two agents has an even stronger antiplatelet effect translating into superior antithrombotic protection in coronary, cerebral or peripheral arterial disease, without an inordinate increase in bleeding complications. Although bleeding episodes are more common with combined antiplatelet therapy than for aspirin alone, the clinical benefit of a significant reduction in morbidity and mortality appears to outweigh the risk of major bleeding in the majority of patients.

A number of seminal clinical trials have demonstrated and confirmed the incremental benefit and efficacy of the combination of clopidogrel and aspirin therapy above and beyond that of aspirin alone, with multiple other important large-scale clinical trials currently ongoing. The exact mechanism of this benefit is still being elucidated but is clearly related to the inhibition of the many consequences of platelet activation--vascular inflammation, endothelial dysfunction, and localized angiogenesis/mitogenesis--and not just aggregation. The combination of clopidogrel and aspirin should be considered in patients with non-ST-segment elevation acute coronary syndrome or those undergoing percutaneous coronary interventions. Particularly in patients undergoing coronary artery stenting, this drug combination has efficiently protected patients from stent thrombosis.

### Acute Coronary Syndromes

Taking into consideration that the most common underlying mechanism of acute coronary syndromes is the development of a non-occlusive platelet-rich thrombus on a disrupted atherosclerotic plaque, it was plausible for investigators to hypothesize that antiplatelet therapy would be the cornerstone therapy in this clinical setting. In this context, several trials have sought to determine whether co-administration of aspirin and clopidogrel, agents that block independent pathways of platelet activation, would offer additive benefit in patients with non-ST elevation acute coronary syndromes. The CURE trial demonstrated that clopidogrel (300 mg loading dose followed by 75 mg daily) in addition to aspirin (doses ranging from 75 to 325 mg) for 3 to 12 months led to a significant 20% relative risk reduction in death from cardiovascular causes, MI or stroke, compared with aspirin alone in patients with acute coronary syndromes without ST-segment elevation (9.3% vs 11.4%,

$p < 0.001$ ) [64]. The benefit derived from clopidogrel was additive to that of aspirin across a wide range of risk subgroups, it was seen as early as 24 hours after randomization and was maintained until the end of the study. Based on the above evidence, the ACC/AHA guidelines recommend the addition of clopidogrel to aspirin as soon as possible on admission in the management of patients with non-ST elevation acute coronary syndromes (NSTEMI/ACS) in whom a non-interventional approach is recommended for at least 1 month (Class IA) and for up to 9 months (Class IB) [129]. In consistency, the 7<sup>th</sup> ACCP conference on anti-thrombotic and thrombolytic therapy-guidelines recommend immediate administration of clopidogrel as bolus therapy (300 mg), followed by 75 mg daily in addition to aspirin in all NSTEMI/ACS patients in whom diagnostic catheterization will be delayed or when coronary bypass surgery will not occur until longer than 5 days following coronary angiography, with an extended duration of co-administration (9 to 12 months) (Grade 1A). Furthermore, placing a relatively high value on avoiding serious bleeding balanced against a low absolute benefit of clopidogrel in the first 24 hours of treatment, clopidogrel administration is suggested to initiate after the coronary anatomy has been determined in NSTEMI/ACS patients in whom angiography will take place rapidly ( $< 24$  h) (Grade 2A) [158].

### **Percutaneous Coronary Interventions (PCI)**

#### ***Bare Metal Stents***

Several randomized controlled clinical trials have demonstrated that after the placement of coronary artery stents, combined antiplatelet therapy with aspirin and clopidogrel has a similar efficacy as aspirin and ticlopidine in preventing major adverse cardiac events including stent thrombosis [159-163]. However, the combination of clopidogrel, including the use of a loading dose, plus aspirin has a superior safety profile and consequently a better cost to benefit ratio compared to that of ticlopidine and aspirin [164]. Thus, the former antiplatelet regimen has become the standard-of-care preventive strategy following stent implantation.

Although non-randomized studies had reported the beneficial effect of thienopyridine pretreatment before percutaneous coronary intervention (PCI), the PCI-CURE trial provided convincing evidence on the hypothesized benefit derived from clopidogrel pretreatment, in patients with acute coronary syndromes, scheduled to undergo a PCI, who were also receiving aspirin. Pretreatment with clopidogrel for a median of 10 days resulted in a 31% reduction in cardiovascular death or myocardial infarction (12.6% vs 8.8%,  $p=0.002$ ). Furthermore, long-term dual antiplatelet treatment after PCI, for a mean period of 8 months, resulted in a significant reduction only in the combined end point of cardiovascular death, myocardial infarction and rehospitalization, compared with the 1-month open label treatment (25.3% vs 28.9%, risk ratio 0.86, confidence interval: 0.74-1.00) [165].

The issue of optimal duration of dual antiplatelet treatment in patients scheduled or highly expected to undergo PCI has been addressed by the CREDO trial which

showed that extending the duration of clopidogrel and aspirin therapy from 28 days to 1 year led to a 26.9% relative risk reduction in the combined risk of death, MI or stroke (8.5% vs 11.5%,  $p=0.02$ ). Furthermore, the same study evaluated the optimal timing of preprocedural administration of combined antiplatelet treatment and demonstrated that only patients treated with a 300 mg loading dose of clopidogrel at least 6 hours before PCI exhibited a significant 38.6% relative reduction in the combined endpoint of death, myocardial infarction or target-vessel revascularization at 28 days [166]. The results of CREDO and PCI-CURE strongly support early (at least 6 hours before PCI) preprocedural loading with clopidogrel in patients scheduled or likely to undergo PCI and emphasize the need for long-term (1-year) post-procedural combined antiplatelet treatment in patients undergoing elective PCI or in patients with acute coronary syndromes managed with an invasive strategy [165,166]. However, pretreatment with clopidogrel in patients with acute coronary syndromes who have not undergone diagnostic coronary angiography and are post-hoc considered as suitable candidates for CABG, necessitates withholding of surgery for at least 5 and preferably 7 days to avoid the increased risk of bleeding during surgery due to clopidogrel administration. In this subgroup of patients, clopidogrel pretreatment is not routinely recommended.

The use of a loading dose in patients undergoing PCI aims at rapidly achieving a clinically effective level of platelet inhibition. A higher loading dose of 600 mg has been shown to result in a more rapid achievement of steady-state levels compared to the conventional loading dose of 300 mg as well as in a maximal antiplatelet effect within 2 hours [95,97]. In a recent randomized controlled clinical trial conducted in low and intermediate risk patients undergoing elective PCI after pre-treatment with a 600-mg loading dose of clopidogrel at least two hours before the procedure, the additional use of the GPIIb/IIIa inhibitor abciximab was associated with no additional measurable benefit [167]. Although the study was not designed to directly evaluate the potential beneficial effect of the higher clopidogrel loading dose, the lower event rate in the placebo group compared to that of the placebo groups in similar controlled trials of other GPIIb/IIIa inhibitors probably reflect the favorable effect of the 600 mg dose [168,169]. However, there is not yet enough evidence to recommend the administration of a higher loading dose on a routine basis. Based on the above mentioned evidence, the 7<sup>th</sup> ACCP conference on anti-thrombotic and thrombolytic therapy-guidelines recommend a loading dose of 300 mg of clopidogrel at least 6 h prior to planned PCI (Grade 1B). If clopidogrel is started  $< 6$  h prior to PCI, a 600-mg loading dose of clopidogrel is suggested (Grade 2C). For PCI patients who cannot tolerate aspirin, the loading dose of clopidogrel (300 mg) should be administered at least 24 h prior to planned PCI (Grade 2C). After PCI, in addition to aspirin, clopidogrel (75 mg/d) is recommended for at least 9 to 12 months (Grade 1A) [158].

#### ***Drug-Eluting Stents***

The optimal duration of antiplatelet therapy after PCI in the case of drug-eluting stenting remains largely undefined.

Several randomized studies have established the superior efficacy of sirolimus-eluting stents in reducing the risk of restenosis, as well as the risk of adverse effects during follow-up mainly due to a lower need for target-lesion revascularization [170-172]. In the RAVEL study [171], patients with single lesions up to 18 mm in length received dual antiplatelet treatment for 2 months, while in the SIRIUS study [172], enrolling patients with more complex lesions, clopidogrel was administered for three months. Patients enrolled in the E-SIRIUS trial, despite their high-risk profile for restenosis, received clopidogrel only for two months, while two patients in the sirolimus-stent group had subacute stent thrombosis compared to none in the control group ( $p=0.25$ ) [170]. In the RESEARCH study [173], which demonstrated the superior efficacy of sirolimus-eluting stents (SES) in consecutive, unselected patients treated in daily practice, compared to bare-metal stents, clopidogrel was prescribed for at least 3 months, unless one of the following was present (in which case clopidogrel was maintained for at least 6 months): multiple SES implantation (>3 stents), total stented length >36 mm, chronic total occlusion, and bifurcations. The dosing regimens of clopidogrel recommended in the abovementioned studies might suggest an underutilization of clopidogrel under clinical conditions where late-stent thrombosis still remains an issue [174].

In a recent trial which showed the superior efficacy of a slow-release, polymer-based, paclitaxel-eluting stent in reducing the risk of clinical and angiographic restenosis

compared to a bare-metal stent, a six-month post-procedural clopidogrel treatment was empirically recommended, while experimental data have demonstrated equivalent rates of endothelialization with slow-release paclitaxel-eluting stents and bare-metal stents [175]. The authors reported that no late stent thrombosis was observed after clopidogrel discontinuation at six months, thus questioning the need of 1-year clopidogrel treatment after the implantation of paclitaxel-eluting stents and recommending 6 to 12 months of clopidogrel after implantation of drug-eluting stents [176]. In this study, patients with complex coronary lesions, such as thrombus containing lesions, bifurcations, calcified stenoses, were excluded from the study. Taking into consideration that complex lesions, which in the real world are the rule rather than the exception, are associated with a higher rate of late thrombosis, a more aggressive preventive antiplatelet strategy should be adopted by clinicians, and consequently, an extended duration of clopidogrel treatment should be reconsidered following drug-eluting stent implantation.

The recently published ACC/AHA guidelines (Table 1) for the management of patients with ST-elevation myocardial infarction make a recommendation for patients who have undergone diagnostic cardiac catheterization and for whom PCI is planned that clopidogrel should be started and continued for at least 1 month after bare metal stent implantation and for several months after drug-eluting stent implantation (3 months for sirolimus, 6 months for paclitaxel) and for up to 12 months in patients who are not at

**Table 1. ACC/AHA/ACCP Recommendations for Aspirin and Clopidogrel Coadministration in Acute Coronary Syndromes (ACS) and Planned Percutaneous Coronary Interventions (PCI)**

STEMI	ACC/AHA (2004) For patients who have undergone diagnostic cardiac catheterization and for whom PCI is planned, clopidogrel should be added to aspirin (75 to 162 mg daily) and continued for at least 1 month after bare metal stent implantation and for several months after drug-eluting stent implantation (3 months for sirolimus, 6 months for paclitaxel) and for up to 12 months in patients who are not at high risk for bleeding ( <i>Class IB</i> ).
NSTEACS	ACC/AHA (2002) In patients in whom a non-interventional approach is recommended, aspirin (162-325 mg followed by 75-160 mg daily) and clopidogrel (300-600 mg loading dose followed by 75 mg daily) should be initiated as soon as possible on admission for at least 1 month ( <i>Class IA</i> ) and for up to 9 months ( <i>Class IB</i> ).  In patients for whom a PCI is planned, clopidogrel should be started and continued for at least 1 month ( <i>Class IA</i> ) and up to 9 months ( <i>Class IB</i> ) in patients who are not at high risk for bleeding
	ACCP (2004) In all patients in whom diagnostic catheterization will be delayed or when CABG will not occur until > 5 days following coronary angiography, clopidogrel should be administered immediately as bolus (300 mg), followed by 75 mg/d for 9 to 12 months in addition to aspirin (75 to 325 mg immediately and then 75 to 162 mg daily) ( <i>Grade IA</i> ). In patients in whom angiography will take place rapidly (24 h), clopidogrel initiation is suggested after the coronary anatomy has been determined ( <i>Grade 2A</i> ).
Planned PCI	ACCP (2004) Pretreatment with aspirin (75 to 325 mg) and a loading dose of 300 mg of clopidogrel at least 6 h prior to planned PCI ( <i>Grade IB</i> ). If clopidogrel is started < 6 h prior to PCI, a 600-mg loading dose is suggested ( <i>Grade 2C</i> ).  After PCI, clopidogrel (75 mg/d) in addition to lower dose aspirin (75 to 100 mg daily) for at least 9 to 12 months ( <i>Grade IA</i> ).  In patients with low atherosclerotic risk (such as isolated coronary lesions), combined antiplatelet administration for at least 2 weeks after placement of a bare metal stent ( <i>Grade IA</i> ), for 2 to 3 months after placement of a sirolimus-eluting stent ( <i>Grade IC</i> ), and 6 months after placement of a paclitaxel-eluting stent ( <i>Grade IC</i> ).

ACC= American College of Cardiology; ACCP= American College of Chest Physicians; AHA= American Heart Association; NSTE= non-ST elevation; PCI= percutaneous coronary intervention; STEMI= ST elevation myocardial infarction

high risk for bleeding (Class IB) [130]. The latter recommendation may be more realistic as newer reports are emerging indicating a continued risk of late stent thrombosis at one year when antithrombotic treatment with aspirin and clopidogrel is discontinued even at such late stage in patients with drug-eluting stents [177].

### **Intracoronary Irradiation (Brachytherapy)**

Intracoronary irradiation (brachytherapy) inhibits cell proliferation, prevents constrictive vascular remodeling and has a documented role in the treatment of in-stent restenosis [174,178-181]. However, intraluminal brachytherapy considerably delays the process of reendothelialization with a subsequent increased risk of late stent thrombosis (>30 days after coronary stenting) [174]. The occurrence of late-stent thrombosis can be substantially reduced by prolonging the use of combined antiplatelet therapy [182]. In the WRIST-PLUS study, 6 months of treatment with clopidogrel and aspirin significantly reduced the overall rate of total occlusion and late stent thrombosis compared to the active gamma-radiation group treated with only one month of antiplatelet treatment [183]; however, in the WRIST-12 study 12 months of aspirin and clopidogrel therapy was superior to 6 months in reducing overall major cardiac events and revascularization rates for patients with in-stent restenosis treated with  $\gamma$ -radiation [184]. Hence, although the optimal period of dual antiplatelet therapy following intracoronary radiation is still largely unknown, the co-administration of aspirin and clopidogrel should be continued for at least 12 months, based on data derived from the WRIST-12 study. Nevertheless, drug-eluting stents appear to have largely supplanted brachytherapy as the principal mode of therapy to combat restenosis.

### **Noncardioembolic Ischemic Stroke**

Results from the CURE [64] and CREDO [166] trials have shown that dual antiplatelet therapy with aspirin and clopidogrel is superior to aspirin treatment alone for prevention of vascular endpoints, with an acceptable increase in bleeding risk, in patients with coronary manifestations of atherothrombosis. Given the substantial differences between the cerebrovascular and the cardiovascular patient population, the extrapolation of these results to the cerebrovascular patients, regarding secondary prevention of ischemic stroke, is prone to inaccuracy. This issue has been directly assessed by the MATCH trial which showed that the addition of low dose aspirin (75 mg) to clopidogrel in high-risk patients with recent ischemic stroke or transient ischemic attack (TIA) failed to significantly reduce the risk of recurrent ischemic vascular events and resulted in a significantly higher risk of life-threatening or major bleeding [185]. Thus, the treatment benefit of combined antiplatelet treatment over clopidogrel alone in secondary prevention of ischemic stroke is rather small and is clearly outweighed by the increased bleeding risk. However, it should be noted that the treatment benefit in the MATCH trial might have been underestimated since patients were enrolled several weeks after the qualifying event and consequently missed the early critical period when the risk of recurrent stroke is highest and the benefit derived by the implemented preventive strategy is maximal

[186,187]. Nevertheless, the abovementioned low risk to benefit ratio of adding even low dose aspirin to clopidogrel treatment in this clinical setting seems to argue against the administration of dual antiplatelet therapy for the secondary prevention of nonembolic ischemic stroke in high-risk patients. The ongoing CHARISMA trial is expected to add further insight in the clinical value of adding clopidogrel to aspirin for high-risk primary prevention and secondary prevention in patients with established cerebrovascular disease [188].

### **Cardioembolic Ischemic Stroke**

Despite the well-established role of warfarin for the primary and secondary prevention of stroke in patients with atrial fibrillation, several therapeutic alternatives have been tested. The background has been largely provided by studies in patients undergoing percutaneous coronary interventions, which showed that combined antiplatelet treatment is associated with an additive antithrombotic effect and an acceptable increase in bleeding complications [64,159-166]. Thus, several studies sought to determine whether the safety profile and efficacy of combined aspirin and clopidogrel were similar to those of warfarin, in patients with atrial fibrillation. A recent phase II, randomized, pilot study demonstrated that dual antiplatelet therapy with aspirin and clopidogrel was equally safe and effective in short-term prevention of thromboembolic complications in non-high-risk patients with permanent or persistent atrial fibrillation awaiting cardioversion [189]. The ACTIVE study, a prospective clinical trial randomizing about 6500 patients with atrial fibrillation to aspirin plus clopidogrel or to warfarin will determine the safety and efficacy of the combined antiplatelet treatment as a preventive strategy in patients with atrial fibrillation [190].

A recent double-blind randomized controlled study has shown that clopidogrel combined with aspirin reduces the postoperative thromboembolic potential in patients undergoing carotid endarterectomy. The rationale of this study was provided by evidence suggesting that preoperative platelet sensitivity to ADP was predictive of postoperative embolization rate [191]. Payne *et al.* showed that a single dose of clopidogrel (75 mg) given the night before surgery to patients on chronic aspirin therapy (150 mg) significantly reduced postoperative embolization, without increasing bleeding complication, but with a significant increase in time needed to secure hemostasis [192]. However, further studies are needed to confirm that the reduction in the surrogate endpoint of postoperative embolization translates into a reduced postoperative thromboembolic stroke rate as well as to standardize the dosing regimen of combined antiplatelet treatment that will provide the ideal cost to benefit ratio in patients subjected to carotid endarterectomy.

### **Adverse Effects of Combined Antiplatelet Therapy**

In the CURE trial [64], patients receiving combined antiplatelet treatment had significantly higher rates of major and minor bleeding, without though an increase in life-threatening bleeding or intracranial hemorrhage. The numbers of patients with thrombocytopenia or neutropenia were similar in the placebo and the clopidogrel group. In the PCI-

CURE, major or life-threatening bleeding rates were similar between the two groups, at 30 days and at the end of follow-up, even in patients who received a GPIIb/IIIa inhibitor. However, minor bleeding episodes were significantly more common in the clopidogrel group compared to the placebo group at the end of follow-up [165]. In the CREDO trial, there was a trend toward an increase in major bleeding in patients treated with clopidogrel for 1 year ( $p=0.07$ ), although the majority of these major bleeding episodes occurred in patients undergoing CABG. On the contrary, there was no difference regarding the incidence of minor bleeding episodes, between the two study groups [166].

This evidence supports the increased risk of bleeding complications conferred by the combined antiplatelet treatment which impairs the cost to benefit ratio. In a post-hoc analysis of the CURE results, Peters *et al.* showed that adding clopidogrel to aspirin exerted a favorable effect in reducing major ischemic events regardless of aspirin dose. Furthermore, the incidence of major bleeding complications significantly increased with increasing aspirin dose. Therefore, using a lower aspirin dose in dual antiplatelet therapy would optimally balance benefit against risk with an optimal dose ranging between 75 and 100 mg in patients with acute coronary syndromes [193]. Finally, a recent study suggests that for patients receiving aspirin, concurrent therapy with a proton-pump inhibitor may be superior to clopidogrel in the prevention of recurrent ulcer bleeding [194].

#### EXPANDED INDICATIONS FOR COMBINED ANTITHROMBOTIC TREATMENT

In many patients with *heart failure*, there is evidence of increased platelet activity related to several factors, which may contribute to the higher risk of clinically manifested thrombotic events in this patient population. The incidence of clinical thromboemboli is estimated to be more than 2% per year in patients with heart failure. The role of antithrombotic therapy to prevent these events has not been examined systematically, but preliminary data from the PLUTO-CHF trial indicate that combined therapy with aspirin and clopidogrel for 1 month provides significantly greater inhibition of platelet activity than aspirin alone in patients with heart failure [195]. Patients with heart failure with heightened platelet activity represent a potential target population in which addition of clopidogrel may decrease mortality rates by reducing the incidence of thrombotic vascular events. Finally, however, the preliminary results of another trial (Warfarin and Antiplatelet Therapy in CHF Trial-WATCH), announced at the AHA meeting in November 2004, failed to show differences between aspirin, warfarin and clopidogrel in the primary endpoint of all-cause mortality, nonfatal MI and nonfatal stroke, although a lower rate of heart failure hospitalizations for warfarin versus aspirin was revealed.

*Atrial fibrillation* constitutes the most common cardiac arrhythmia, affecting approximately 1% of the general population and is associated with serious thromboembolic complications, most notably ischemic stroke, occurring at an annual rate of 4-5%. Anticoagulation therapy with warfarin has been demonstrated to reduce the risk of stroke by 60-

70%, but warfarin therapy is markedly underused in clinical practice because of its narrow therapeutic window and its implications on quality of life. Combined antiplatelet therapy with aspirin and clopidogrel has been recently shown to be protective against thrombotic events related to blood stasis. Accordingly, an ongoing randomized controlled trial, with the acronym of ACTIVE (Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events) has been designed to vigorously examine the role of combined antithrombotic therapy for prevention of vascular events, including stroke in high-risk patients with atrial fibrillation [196].

In patients undergoing demanding *catheter ablation* procedures to effect nonsurgical cure of cardiac arrhythmias, there is evidence of increased prothrombotic activity incurred by the application of radiofrequency energy to intracardiac sites [197]. Thromboembolic complications have been reported to occur at a rate of 1-2% after such procedures [198,199]. More devastating are the thromboembolic events occurring during ablation in the left heart, which usually involves patients of younger age. Preliminary data indicate that pretreatment with combined antiplatelet agents, such as aspirin and ticlopidine, the latter now supplanted by clopidogrel, might provide a protective effect from thromboembolic events [200,201]. Thus, in addition to using intravenous heparin during these procedures, a 3-day pre-treatment regimen with combined aspirin and clopidogrel might prove beneficial to patients undergoing radiofrequency ablation, particularly in those submitted to pulmonary vein ablation for paroxysmal atrial fibrillation. Such therapy might be continued for 1 month after right-sided procedures or for 3 months for ablation performed in the left heart, whereby cases of thromboembolism have been reported even up to 3 months after the procedure. Newer data from future randomized trials are needed to more widely adopt such a recommendation.

#### CONCLUSION

Atherothrombotic coronary artery disease is the single most common cause of death worldwide and a growing public health problem. Platelets play a pivotal role in the pathogenesis of atherothrombosis, believed to be integrally involved in both the development and progression of atherosclerotic heart disease, as well as in its acute thrombotic complications. Intracoronary thrombi produced during atherosclerotic plaque rupture spontaneously or mechanically during PCI result from activation and aggregation of platelets. Platelets are therefore commonly targeted by antiplatelet drugs as part of strategies of primary and secondary prevention of atherothrombosis. Antiplatelet therapy constitutes the cornerstone in the management of patients with acute coronary syndromes and generally high-risk patients with atherothrombosis.

Until recently, long-term antiplatelet therapy for the treatment and prevention of the complications of atherothrombotic disease was traditionally limited to aspirin. The availability of the thienopyridines, in particular clopidogrel, represents an important addition to the physician's armamentarium. Aspirin and clopidogrel interfere with platelet activation in complementary, but separate pathways. Both

**Table 2. Clinical Studies Supporting the Use of Combined Antiplatelet Therapy in Cardiovascular Disease**

Trial	Study design	Primary endpoint	Incidence %		P	Relative risk (95% CI)
			Treatment Group	Control group		
CURE	<i>NSTEACS</i> Clopidogrel (300 mg, followed by 75 mg qd) or placebo plus aspirin for 3 to 12 months	composite of death from CV causes, nonfatal MI or stroke	9.3%	11.4%	<0.001	0.80 (0.72-0.90)
PCI-CURE	<i>NSTEACS patients undergoing PCI</i> Clopidogrel or placebo pretreatment plus aspirin before PCI followed by open label thienopyridine for about 4 weeks	composite of CV death, MI, or urgent target-vessel revascularisation	4.5%	6.4%	0.03	0.70 (0.50-0.97)
CREDO	<i>Planned or likely PCI</i> Clopidogrel (300mg) or placebo before PCI. After stenting open label clopidogrel for 28 days. After 28 days 1-year clopidogrel in the pre-treatment group vs placebo in the non-pretreatment group	1-year incidence of the composite of death, MI or stroke	8.5%	11.5%	0.02	0.73 (0.56-0.96)
MATCH	<i>Recent ischemic stroke or TIA</i> Aspirin (75mg daily) plus clopidogrel (75mg daily) vs clopidogrel (75mg daily) for 18 months	composite of ischemic stroke, MI, vascular death, or rehospitalization for acute ischemia	15.7%	16.7%	0.244	0.94 (0.84-1.05)

CV= cardiovascular; MI= myocardial infarction; NSTEACS= non-ST elevation acute coronary syndrome; PCI= percutaneous coronary intervention; TIA= transient ischemic attack

these antiplatelet agents have a potent protective effect against adverse vascular events, but the combination of these two agents has an even stronger antiplatelet effect translating into superior antithrombotic protection in coronary, cerebral or peripheral arterial disease, without an inordinate increase in bleeding complications. Although bleeding episodes are more common with combined antiplatelet therapy than for aspirin alone, the clinical benefit of a significant reduction in morbidity and mortality appears to outweigh the risk of major bleeding in the majority of patients. Cardiological and other societies have put forth guidelines and recommendations for the use of combined antiplatelet therapy (Table 1). These guidelines are based on a number of seminal clinical trials which have demonstrated and confirmed the incremental benefit and efficacy of the combination of clopidogrel and aspirin therapy above and beyond that of aspirin alone (Table 2), with multiple other important large-scale clinical trials currently ongoing.

Newer data are being accumulated from studies where indications for the use of clopidogrel and aspirin continue to expand into other patient groups, rendering this dual antiplatelet drug therapy a sweeping combination in Cardiology. However, important issues remain to be further and more thoroughly explored about the benefit of this antiplatelet drug combination in these other patient groups, such as in patients with *heart failure*, where preliminary data indicate a favorable effect on thrombotic vascular events, in patients with *atrial fibrillation*, where there is hope that this

combination may replace or be an alternative treatment modality to coumadin in certain subpopulations, in patients undergoing demanding *catheter ablation* procedures, where data point to a protective effect from thromboembolic events. Another pertaining issue to be further investigated is the occurrence of *drug-resistance* observed in some patients for both these antithrombotic agents.

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