Review Article

Resistance to Aspirin and Clopidogrel: Possible Mechanisms, Laboratory Investigation, and Clinical Significance

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The efficacy of aspirin and clopidogrel treatment in patients with coronary artery disease is well documented. However, recent studies that investigated the *in vitro* response of platelets to the administration of these drugs showed this response to be variable. Some patients have been observed to exhibit nonresponsiveness or resistance, thus having an increased risk of thrombotic episodes. So far, there is no generally accepted method for the *ex vivo* evaluation of platelet reactivity following aspirin or clopidogrel administration or for assessing the extent of platelet inhibition by these two drugs. For this reason, it is not possible to set out specific guidelines for the treatment of patients who show high platelet reactivity or poor platelet inhibition after treatment with aspirin or clopidogrel. The aim of this review article is to present data from laboratory and clinical studies that are related to resistance to aspirin or clopidogrel, and to discuss the possible causes, the clinical significance, and the ways of managing such resistance at the clinical level.

The role of platelets in acute coronary syndromes

Platelets play an important role in the pathogenesis of atherothrombosis. The interactions among platelets, atherosclerotic lesions, and the coagulation system comprise the main underlying pathophysiological mechanism for the occurrence of an acute coronary syndrome in patients with asymptomatic coronary artery disease or stable angina. Disruption of the intima of a coronary artery wall, which may occur either spontaneously during rupture of an atherosclerotic plaque, or iatrogenically during a percutaneous coronary intervention, results in the exposure of subendothelial components, such as von Willebrand factor and collagen, to the intravascular space. These substances are then recognised by specific receptors in the platelet membrane (glycoprotein receptors Ia/IIa and Ib/V/IX, respectively), leading to platelet adhesion to the subendothelial area at sites of endothelium injury. The platelet adhesion in its turn induces the activation of a cascade of intracellular metabolic signalling pathways, with the final result being the further activation and aggregation of platelets together with the activation of clotting and inflammatory mechanisms.[1,2]

Platelet activation causes both structural and functional changes in these cells.

Among these, an important role is played by the change in the conformation of platelet integrin receptor $\alpha_{IIb}\beta_3$ (glycoprotein [GP] IIb/IIIa]) in the platelet membrane. The conformational changes undergone by this receptor result in its binding with fibrinogen, leading to platelet aggregation via fibrinogen bridges. This, in combination with the adhesion of platelets to the subendothelial region, has as final result the creation of platelet thrombus at the site of the vascular lesion. Platelet aggregation is also associated with a change in their shape. The altered platelet shape provides the necessary surface area to support the activation of the coagulation cascade. Apart from this, platelet activation also results in the secretion of various bioactive substances by the α- and dense granules. Some of these, such as adenosine diphosphate (ADP), bind to specific receptors on the cell membrane of adjacent platelets, increasing their activation further. Platelet activation causes the release of arachidonic acid from the phospholipids of the cell membrane via the action of a cytosolic phospholipase A_2. In the platelets arachidonic acid is mainly metabolised to prostaglandins G2/H2 by the action of the cyclooxygenase enzyme (COX-1), whence it sub-

sequently forms thromboxane A_2 (TxA_2) through the action of TxA_2 synthase enzyme (Figure 1). TxA_2 further activates the platelets via a special receptor, while at the same time it exerts various actions on cells of the arterial wall, contributing to the pathophysiological mechanisms of atherothrombosis.[3]

As stated above, among the various platelet agonists ADP plays an important role. ADP activates platelets by binding to purinergic receptors $P2X_1$, $P2Y_1$ and $P2Y_{12}$ on the platelet membrane. These receptors act synergistically in platelet activation and aggregation. More specifically, $P2X_1$ is involved in the change in platelet shape, $P2Y_1$ activation participates in the initial, reversible activation of platelets, and activation of $P2Y_{12}$ contributes to the prolonged activation and aggregation of platelets (Figure 2).[4]

Pharmacological action and pharmacokinetics of aspirin and clopidogrel

Aspirin, acetylsalicylic acid, is the salicylic ester of acetic acid, and is the product of the acetylation of salicylic acid by acetic anhydride. The absorption of aspirin by the stomach and duodenum is rapid and al-
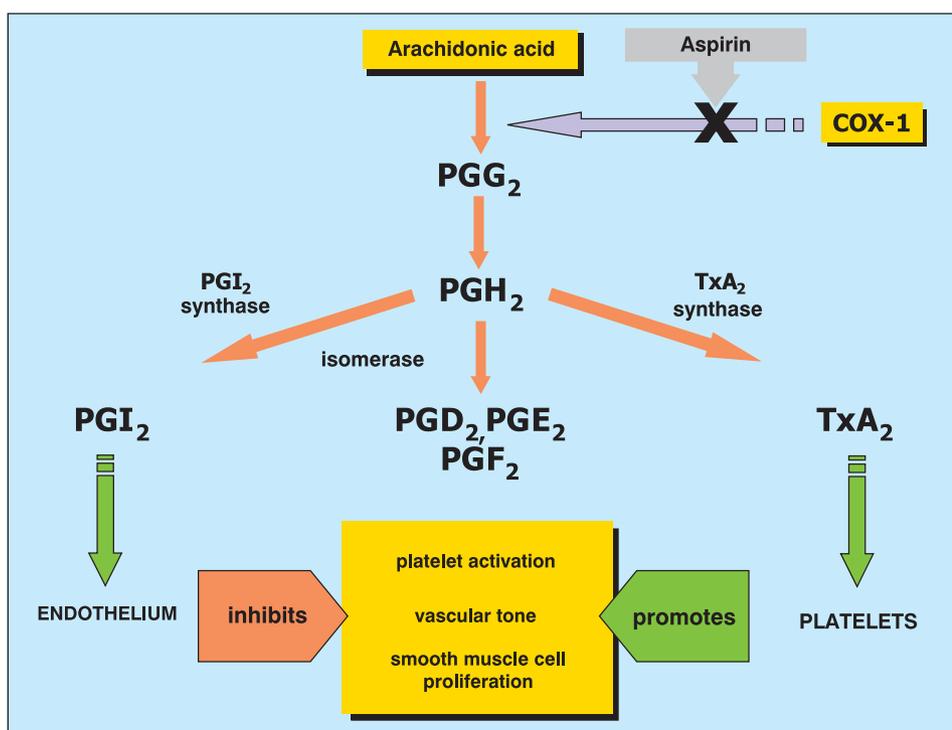
Figure 1. Metabolism of arachidonic acid in platelets and its inhibition by aspirin. COX-1 – cyclooxygenase-1; PGD_2, PGE_2, PGF_2, PGG_2, PGH_2, PGI_2 – prostaglandins D_2-I_2, respectively; TxA_2 – thromboxane A_2.
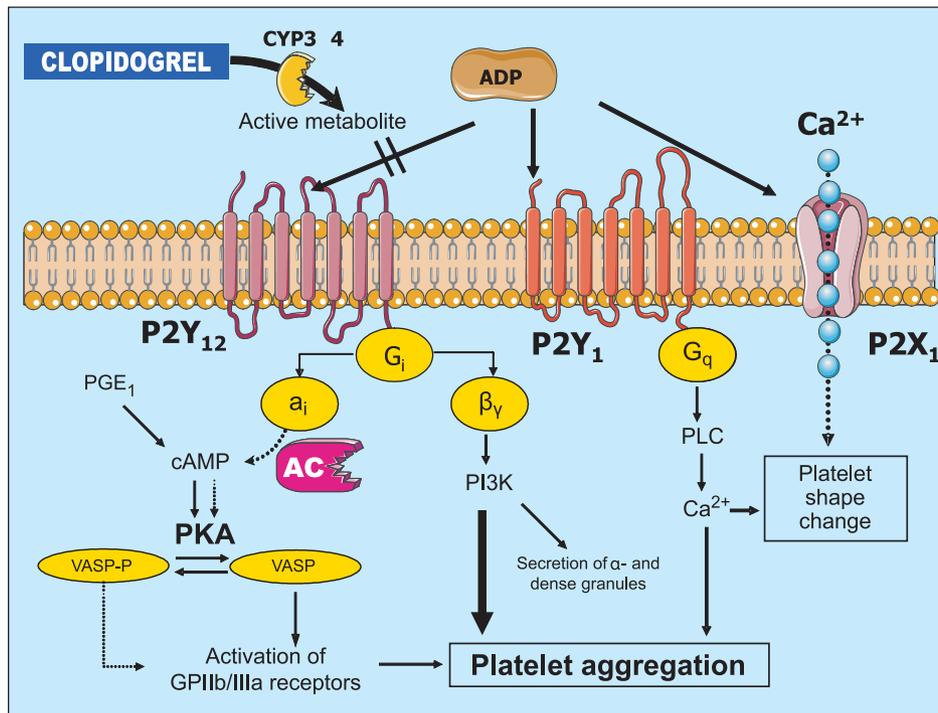
Figure 2. Mechanism of clopidogrel action on platelets. AC – adenyl cyclase; ADP – adenosine diphosphate; cAMP – cyclic adenosine monophosphate; CYP3A4 – cytochrome P450 4A4 in the liver; $P2Y_{12}$, $P2Y_1$, $P2X_1$ – purinergic receptors for ADP; PI3K – phosphatidylinositol 3-kinase; PKA – protein kinase A; PLC – phospholipase C; VASP – vasodilator stimulated phosphoprotein; VASP-P – phosphorylated form of VASP

most total, since 80-100% of the drug is absorbed within 20 minutes to 2 hours following the oral ingestion of non-enteric coating tablets. In contrast, enteric coating tablets show significantly less absorption, resulting in a 40-50% lower bioavailability of the drug in the blood for more than 3-8 hours after oral administration.[5] The half life of aspirin in the blood is short (15-30 minutes) because of rapid hydrolysis of the drug in the intestinal mucosa, the liver, and the blood. For this reason, it is likely that aspirin's antiplatelet action involves mainly the platelets in the portal circulation, namely, before its hydrolysis in the liver.[5] Although various mechanisms have been proposed for the inhibitory action of aspirin on platelet activation, most researchers agree that the main mechanism of aspirin's antiplatelet action consists in its ability to acetylate irreversibly the hydroxyl group of a serin at site 529 of the catalytic centre of the COX-1 molecule, resulting in the inhibition of the binding of arachidonic acid to the catalytic centre of the enzyme, an essential stage for its further metabolism and the formation of TxA_2.[6,7]

For the complete inhibition of platelet aggregation by aspirin, it is necessary to inhibit TxA_2 production by >90%. In most patients the dosage of aspirin required to accomplish this can be as high as 30 mg daily.[8] Platelets are anucleate. Therefore, when they are exposed to aspirin COX-1 is deactivated and remains inactive for the remaining lifespan of the cell, namely 7-10 days, since these cells are unable to synthesise new, active COX-1. For this reason the restoration of normal platelet function after aspirin administration occurs only with the production of new platelets by the bone marrow. It should be noted that 1/7 of the platelets in the circulation are renewed every 24 hours; therefore up to 30% of circulating platelets may show normal TxA_2 production after aspirin administration has been discontinued for 48 hours. In view of this, aspirin administration on a daily basis should be preferred rather than administration every second day.[9] It must be stressed that in low doses aspirin does not affect the action of endothelial cell COX-1 and therefore does not reduce the production of prostaglandin I_2 (PGI_2), which has many beneficial effects (Figure 1). In consequence, low doses of aspirin do not affect renal function and do not decrease

the antihypertensive action of diuretics or angiotensin converting enzyme inhibitors.[10,11]

Clopidogrel (clopidogrel hydrogen sulphate, or methyl (+)-(S)-alpha-(o-chlorophenyl)[6,7]-dihydro-thieno [3,2-c]pyridine-5(4H)-acetate hydrogen sulphate), is a thienopyridine derivative. Clopidogrel is a pro-drug that is converted in the liver to its pharmacologically active metabolite, mainly by the action of cytochrome P450 3A4 (CYP3A4). Cytochromes CYP3A5 and CYP2B6 also participate in the metabolism of clopidogrel and the formation of its active metabolite, but to a significantly lesser extent than CYP3A4.[12,13] Peak levels of the active metabolite in the blood are seen within one hour after the oral administration of 600 mg clopidogrel.[13] It should be noted that only a small part of the drug is converted to active metabolite, while 85% is hydrolysed, forming products without any biological action.[13] About 50% of the clopidogrel administered is excreted in the urine and 46% in faeces. The half life of the active metabolite after isolated or repeated administration of clopidogrel is 8 hours. The daily administration of 75 mg clopidogrel without a previous loading dose leads to peak inhibition of platelet activation within 3-7 days, while platelet function is restored at least 5 days after cessation of medication.

The active metabolite of clopidogrel is a powerful selective inhibitor of the ADP purinergic receptor $P2Y_{12}$ and causes reversible inhibition of the binding of ADP to that receptor. The active metabolite of clopidogrel forms disulphide bonds with two residual serins at sites 17 and 270 (Ser17 and Ser270) on the $P2Y_{12}$ receptor molecule, resulting in its non-reversible deactivation.[14] The inhibition of the binding of ADP to receptor $P2Y_{12}$ by clopidogrel's metabolite results in an increase in levels of cyclic adenosine monophosphate (c-AMP) inside the platelets, which among other things causes phosphorylation of vasodilator stimulated phosphoprotein (VASP), eventually leading to inhibition of the activation of GPIIb/IIIa receptors and consequently to prevention of platelet aggregation (Figure 2).[15] Apart from aggregation, clopidogrel also inhibits ADP-induced expression of P-selectin and CD40L in platelet membrane, while at the same time it reduces the interactions between platelets and leukocytes, which play an important role in the formation of thrombus and the complete occlusion of the vascular lumen as a consequence of the rupture of atherosclerotic plaque.[16]

The efficacy of aspirin and clopidogrel in the treatment of patients with coronary artery disease

The efficacy of aspirin administration in improving cardiac morbidity and mortality in patients with proven atheromatous disease has been studied in a large number of randomised, multi-centre studies.[17] Taken together, the results of these studies were presented in a meta-analysis[8] which showed that aspirin administration causes a 25% reduction in total events. Specifically, low-dose administration of aspirin led to a reduction in the incidence of fatal myocardial infarction by 37%, of ischaemic stroke by 25%, and of deaths from vascular or other causes by 16%. The clinical benefits from aspirin administration were in direct proportion to the overall cardiovascular risk; that is, the greater the annual cardiovascular risk the greater the benefit from aspirin administration.[8] Based on these data, the administration of 75-100 mg is recommended for the long-term prevention of cardiovascular episodes in high-risk patients, such as those with documented atheromatous disease and a history of coronary or vascular disease, or those with diabetes mellitus.

The efficacy of aspirin for primary prevention in low-risk patients is not clear, since the relatively low benefit (1-2% per year) from administration of the drug is offset by the risk of significant haemorrhage (also 1-2% per year).[5] In contrast, the risk/benefit ratio improves when aspirin is given to patients with a high cardiovascular risk. It is also likely that the efficacy of aspirin administration for primary prevention is sex-related. In the Women's Health Study,[18] the administration of low doses of aspirin (100 mg every second day) to low-risk women compared to a placebo control group resulted in a reduction of the risk of an acute ischaemic vascular event by 17%. In the same study, aspirin administration did not reduce the risk of acute myocardial infarction.[18] In contrast, in healthy men aged over 45 years aspirin administration resulted in a reduction in the risk of acute myocardial infarction by 32%, without any corresponding reduction being observed in the risk of an acute ischaemic vascular event.[19,20] These results were confirmed by a very recent meta-analysis that included a total of 51,342 patients.[21] In that meta-analysis it appeared that aspirin administration for primary prevention resulted in a reduction in total cardiovascular events, which in men was due to a reduction in acute infarctions, whereas in women it was due to a reduction in ischaemic stroke.[21]

The efficacy of clopidogrel administration in improving cardiovascular morbidity and mortality in patients with acute coronary syndromes has been demonstrated during the last decade by the results of various multi-centre studies. Monotherapy with clopidogrel has been shown to be effective in patients with established coronary artery disease, having safety levels comparable to those of aspirin.[22,23] In the CAPRIE trial, the administration of 75 mg clopidogrel resulted in a 1% reduction in the absolute risk for myocardial infarction, stroke, or cardiovascular death, compared with 325 mg aspirin.[22] The results of that trial established clopidogrel as an alternative monotherapy in the treatment of patients with coronary artery disease. Furthermore, clopidogrel administration in combination with aspirin reduces the mortality and morbidity rates of patients following an acute myocardial infarction without persistent ST-segment elevation (NSTEMI).[24] Similar results were reported from the combined administration of aspirin and clopidogrel to patients who had an acute myocardial infarction *with* persistent ST-segment elevation (STEMI),[25,26] as well as from administration to patients after angioplasty.[23,27]

The role of the coadministration of aspirin and clopidogrel to high-risk patients was studied recently in the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance) trial.[28,29] CHARISMA was a randomised, double-blinded, multi-centre study that was designed to investigate the efficacy of long-term administration of the combination of aspirin and clopidogrel in the prevention of cardiovascular events in a wide spectrum of patients with high cardiovascular risk. A total of 15,603 patients were enrolled and were randomised to receive either low-dose aspirin and 75 mg/day clopidogrel, or low-dose aspirin and placebo. The mean duration of follow up was 28 months. Primary endpoints of the study were death from cardiovascular cause, non-fatal myocardial infarction, and non-fatal ischaemic stroke. During the follow-up period 1107 patients in all presented a primary endpoint. There were no significant differences between the two study groups in the incidence of primary endpoints.[30] In contrast, the incidence of a secondary endpoint (first acute myocardial infarction, stroke, cardiovascular death, hospitalisation for unstable angina or transient ischaemic cerebrovascular event or coronary reperfusion intervention) was significantly lower in the clopidogrel group (16.7% versus 17.9%, p=0.004).[30] It should be noted that when the patients were divided into "symptomatic" (patients with documented cardiovascular disease) and "asymptomatic" (patients with multiple risk factors but without documented cardiovascular disease) the subgroup analysis showed that in the "symptomatic" patients, namely those at higher risk, clopidogrel administration was associated with a lower incidence of primary endpoints (6.9% versus 7.9%, p=0.046), while in the "asymptomatic" group there was a higher incidence of cardiovascular deaths in the clopidogrel patients (3.9% versus 2.2% for placebo, p=0.01).[30]

The results of the above trials confirmed those of other clinical studies, with the result that the coadministration of aspirin and clopidogrel is the preferred therapeutic strategy in the acute phase of a coronary syndrome (with or without persistent ST-segment elevation), and is recommended by both the 2004 Conference of the American College of Chest Physicians[5,31,32] and the European Society of Cardiology.[33,34] In contrast, dual antiplatelet medication is not recommended for primary prevention of atherothrombotic disease. In the guidelines of the Hellenic Cardiological Society that were published recently,[35] the administration of aspirin and clopidogrel is recommended in patients with an acute coronary syndrome (with or without persistent ST-segment elevation) and in patients who are to undergo percutaneous coronary angioplasty. The dosages of the two drugs depend on the clinical syndrome, the duration of administration, and the therapeutic strategy that is determined for each individual patient.[35]

Resistance to aspirin and clopidogrel

Many studies in recent years have demonstrated the phenomenon of resistance to the action of aspirin or clopidogrel in patients with cardiovascular disease or stroke.[36-42]

Resistance to aspirin

Aspirin resistance may be categorised as either 'laboratory' or 'clinical'. Laboratory aspirin resistance is defined as the failure of aspirin to inhibit the production of TxA_2 by platelets or to inhibit the platelet activation that depends on TxA_2 production. Clinical aspirin resistance is defined as the failure to prevent the occurrence of atherothrombotic ischaemic episodes in patients to whom it is administered.[43]

Clinical aspirin resistance has been observed in 5-9% of patients with cardiovascular disease, while partial resistance to the drug has been observed in a further 23%. It has also been demonstrated that 35% of patients with new cerebrovascular stroke show laboratory aspirin resistance. In addition, the incidence of cardiovascular events in patients with laboratory aspirin resistance is 40%, compared with 4.4% in patients who respond to the drug.[44] The possible pathophysiological mechanisms responsible for aspirin resistance are as follows (summarised in Table 1).

Reduced inhibition of platelet COX-1

1. Insufficient aspirin dosage.
2. Interaction between aspirin and other drugs, as happens with the simultaneous administration of non-steroid anti-inflammatory drugs, which prevents the access of aspirin to the COX-1 binding site.
3. Increased production of new, immature forms of platelet, which are produced during the intervals between aspirin doses and have not come into contact with the drug.
4. Genetic polymorphism of COX-1.

Bypassing of the inhibitory action of aspirin on platelet COX-1

1. Alternative sources of TxA_2 apart from platelets, such as monocytes, macrophages, and endothelial cells, via the action of COX-2 enzyme, which is not blocked by aspirin.
2. Increased production of new, immature forms of platelet with elevated COX-2 levels.

Reduced bioavailability of aspirin

1. Insufficient uptake of the drug (poor compliance).[45]
2. Insufficient dosage.
3. Reduced absorption or increased catabolism of aspirin.

Alternative routes of platelet activation

1. Platelet activation via routes that are not blocked by aspirin (activation by collagen, ADP, epinephrine, thrombin, etc.).
2. Increased platelet sensitivity to the action of collagen and ADP.

Increased platelet production

1. Increased production of platelets by the bone marrow in response to stress conditions (e.g. after aortocoronary bypass), resulting in the entry of new platelets to the circulation in the intervals between aspirin doses so that they do not come into contact with the drug.

Genetic polymorphisms

1. Polymorphisms that involve COX-1, COX-2, TxA_2 synthase or other enzymes that participate in the process of arachidonic acid metabolism.
2. Polymorphisms that involve GP receptors Ia/IIa, Ib/V/IX, as well as collagen and von Willebrand factor receptors.
3. The Val34Leu polymorphism of factor XIII, which results in variable inhibition of its activation by low doses of aspirin.

Reduction in antiplatelet action of aspirin when administered over a long period of time

1. Tachyphylaxis.

Vascular episodes of non-atherothrombotic aetiology

1. Non-cardiac embolic episodes (red fibrin thrombi, vegetations, calcifications, tumours, prosthetic valves).
2. Arteritis.

Table 1. Mechanisms of resistance to aspirin and clopidogrel.

Mechanisms of aspirin resistance:
- Reduced inhibition of platelet COX-1
- Bypassing of the inhibitory action of aspirin on platelet COX-1
- Reduced bioavailability of aspirin
- Alternative routes of platelet activation
- Increased platelet production
- Genetic polymorphisms
- Reduction in antiplatelet action of aspirin when administered over a long period of time
- Vascular episodes of non-atherothrombotic aetiology

Mechanisms of clopidogrel resistance:
- Patients' non-compliance
- Reduced bioavailability
- Variable clearing of clopidogrel's active metabolite
- Alternative routes of platelet activation
- Genetic polymorphisms
- Increased ADP secretion
- Changes in clopidogrel metabolism via CYP3A4

Resistance to clopidogrel

Clopidogrel resistance is related with the degree of inhibition of platelet activation by ADP *in vitro* in patients who are under clopidogrel administration.[46] The response of platelets to clopidogrel shows a wide variation between different individuals, and for this reason some authors describe clopidogrel resistance as variability in its action.[47,48] The platelet response to clopidogrel is studied mainly through the determination of the difference in platelet aggregation in ADP before and after the drug is administered. A reduction in platelet ADP aggregation by <10% in response to clopidogrel administration is considered as an absence of antiplatelet action and these patients are characterised as clopidogrel resistant.[49] The variability in patients' response to clopidogrel has been confirmed by many studies. The incidence of non-response to clopidogrel in those studies ranged from 4% to 44%.[50] This wide range is due to the laboratory methods used (different methods or different concentrations of agonists) and to the criteria used to define resistance or reduced response to clopidogrel.[51]

The possible underlying pathophysiological mechanisms for clopidogrel resistance are as follows (summarised in Table 1).

1. Patients' non-compliance or administration of insufficient dosage of the drug, leading to reduced bioavailability.
2. Variability in clearing of clopidogrel's active metabolite.
3. Increased regulation of platelet activation mechanisms by the action of ADP.
4. Increased expression of $P2Y_{12}$ receptors on the surface of platelets as the result of genetic polymorphism.
5. Increased secretion of ADP by the dense granules of platelets.
6. Variability in clopidogrel metabolism and the production of its active metabolite via CYP3A4.

Here it should be mentioned, in relation to the metabolism of clopidogrel via CYP3A4, that various studies showed initially that the coadministration of clopidogrel and lipophilic statins that are metabolised via CYP3A4 resulted in blockade of clopidogrel's antiplatelet action, at least in the short term follow up.[52] Subsequently, however, results of independent studies failed to confirm the initial reports.[53-56] Additional

post hoc analysis of data from the CREDO (Clopidogrel for Reduction of Events During Observation) trial showed that the coadministration of lipophilic statins and clopidogrel was not associated with a worse outcome in patients who underwent coronary artery angioplasty.[57] It is thus accepted nowadays that the antiplatelet action of clopidogrel is not affected by the coadministration of various doses of atorvastatin, or other lipophilic statins such as simvastatin and lovastatin.[35]

Laboratory methods for the evaluation of resistance to aspirin and clopidogrel

Aspirin resistance may be evaluated in the laboratory either directly, by measuring TxA_2 production, or indirectly, by assessing the percentage platelet activation via mechanisms related to TxA_2.[58] TxA_2 production can be measured by measuring its stable metabolites in the plasma, namely serum TxB_2 and 11-dehydro-TxB_2 in the urine. Since TxB_2 levels are governed mainly by platelet COX-1, their determination is a reliable index of the efficacy of the antiplatelet action of low doses of aspirin.[58] Methods of estimating the sensitivity of platelets to the aggregative action of TxA_2 include arachidonic acid-induced platelet aggregation measured by light or optical transmission (turbidimetric aggregometry in platelet rich plasma), electrical impedance (whole blood platelet aggregometry), or semi-automated platelet aggregometry, such as platelet function analyser PFA-100, or the Ultegra rapid platelet function assay (RPFA). The determination of the bleeding time is a method for the *in vivo* evaluation of platelet activation. Bleeding time depends to a large degree on TxA_2 production by activated platelets, but the method is used only rarely because the determination is highly operator dependent and has very low reproducibility.[58] Other laboratory methods for assessing aspirin resistance include Plateletworks (Helena Laboratories, Beaumont, TX, USA), the IMPACT analyser (Diamed, Cressier, Switzerland), thromboelastography, and the use of flow cytometry for the evaluation of activation-dependent changes on platelet surface, such as P-selectin or GPIIb/IIIa expression, or to determine leukocyte-platelet aggregates.[59]

Clopidogrel resistance can be estimated in the laboratory by determining platelet aggregation by ADP, using the techniques described above. Activa-

tion of platelets by ADP may also be determined using flow cytometry. Finally, clopidogrel resistance can be assessed by determining VASP protein phosphorylation, using flow cytometry and a monoclonal antibody that specifically recognises the phosphorylated form of VASP.[59]

Clinical significance of resistance to aspirin and clopidogrel

Given that aspirin reduces cardiovascular risk,[7,8,10,11,21,35,60,61] aspirin resistance is likely to reduce the value of its administration. To date, studies that assessed the clinical significance of aspirin resistance are few, usually retrospective, often lacking the necessary statistical power, while the definition of aspirin resistance was unclear and differed from study to study.

It should be noted that some studies showed a worse prognosis in patients with aspirin resistance; however, that conclusion was not confirmed by others. Thus, in a subanalysis of the HOPE study, involving 976 patients under treatment with aspirin, the risk of occurrence of myocardial infarction or stroke or cardiovascular death during a 5-year follow up period increased with each greater quartile of urine levels of 11-dehydro-TxB$_2$.[62] In contrast, in a prospective, multi-centre study including 289 patients who were followed for two years after programmed aortocoronary bypass, no significant differences were observed in the risk of thrombotic episodes between patients who responded to aspirin and those who had aspirin resistance, as determined by bleeding time.[63] Consequently, data regarding the clinical incidence of aspirin resistance are scarce and it would still be unsafe to draw any conclusions.

There are limited data concerning the correlation between clopidogrel resistance and the risk of new thrombotic episodes. A number of studies have investigated the correlation between platelet sensitivity to the action of ADP before the start of therapy and clopidogrel resistance. The results of those studies were conflicting; thus, the estimation of the risk of thrombotic episodes through the determination of the above parameters is considered unreliable and likely to over- or underestimate the numbers of patients concerned. Based on these observations, the view subsequently emerged that determination of platelet sensitivity to the action of ADP after clopidogrel administration is probably a more reliable prognostic index for future thrombotic events.[64] Platelet sensitivity to ADP after

clopidogrel administration was investigated in a study that evaluated the degree of platelet activation in patients under chronic clopidogrel treatment who underwent non-emergency angioplasty. The results showed that the increased risk of a cardiovascular event 6 months after the procedure was directly related to the increased platelet activation caused by ADP.[65] Further studies showed that a high platelet response to the action of ADP in patients who are taking clopidogrel may be an independent risk factor for in-stent thrombosis.[38,66] In the CREST study,[67] the degree of platelet activation was assessed in patients with or without stent thrombosis. Platelet activation was determined by aggregometry, from the expression of GPIIb/IIIa receptors, and from VASP phosphorylation following ADP activation. Increased levels of all three parameters were seen in the patients who had in-stent thrombosis, suggesting that the thrombosis was related to inadequate blockade of the platelet P2Y$_{12}$ receptor by clopidogrel.[67] To summarise, the results of the above studies indicate that inadequate blockade of the platelet P2Y$_{12}$ receptor and increased platelet activation in spite of clopidogrel therapy are directly related with an increased risk of in-stent thrombosis and repeated ischaemic episodes in patients who have undergone coronary angioplasty.

Management of resistance to aspirin and clopidogrel

In the case of aspirin resistance, it is reasonable that the best way of reducing the risk of repeated ischaemic episodes consists in the correct recognition and the prompt treatment of the underlying causes to which the condition is due. In patients whose new ischaemic episode is of non-atherothrombotic origin, recognition and appropriate treatment of the underlying cause (antibiotics for endocarditis, steroid administration for arteritis) is considered sufficient. Additional measures that have been proposed include the improvement of the patient's compliance with therapy, avoidance of the coadministration of drugs antagonistic to the action of aspirin (e.g. ibuprofen), cessation of smoking, an increase in the dosage and frequency of aspirin administration, and the replacement or supplementation of aspirin therapy with antiplatelet drugs that inhibit alternative routes of platelet activation (ADP receptor blockers, TxA$_2$ receptor antagonists), or the common route of platelet aggregation (intravenous administration of GPIIb/IIIa receptor blockers). It must be stressed, however,

that the efficacy and safety of these measures have yet to be documented. Despite the failure of treatment in a proportion of patients, aspirin is still the most widely administered drug and the one with the best cost-benefit ratio in the secondary prevention of cardiovascular disease.[36]

Regarding clopidogrel resistance, the results of recent clinical studies showed that the administration of 600 mg instead of 300 mg clopidogrel as a loading dose in patients undergoing angioplasty with stenting was associated with a higher level of inhibition of platelet activation and a lower incidence of non-response to clopidogrel's action.[68,69] In the ISAR-CHOICE trial it was observed that the concentrations of both clopidogrel and its active metabolite in the serum, as well as the inhibition of platelet activation, reached their peak when a 600 mg loading dose of clopidogrel was given. Administration of a higher dose, 900 mg, was not associated with any further increase in either blood concentrations or platelet inhibition. However, observations based on the pharmacokinetics of both clopidogrel and its metabolite led to the conclusion that the variability in the organism's response to clopidogrel was probably mainly due to peculiarities in the absorption of the drug by the intestinal mucosa.[70] For this reason, patients with elevated platelet sensitivity to ADP are likely to benefit from higher doses of clopidogrel. The validity of this hypothesis needs to be proved by large, randomised, clinical studies. Nevertheless, and despite the specific limitations, the recent guidelines of the American College of Cardiology and American Heart Association (ACC/AHA) for the treatment of patients undergoing coronary angioplasty include the class IIa recommendation that "a regimen of greater than 300 mg is reasonable to achieve higher levels of antiplatelet activity more rapidly."[51] Finally, the same guidelines include the class IIb recommendation that "in patients in whom subacute thrombosis may be catastrophic or lethal, platelet aggregation studies may be considered and the dose of clopidogrel increased to 150 mg/day if less than 50% inhibition of platelet aggregation is demonstrated."[51] An alternative solution for the treatment of clopidogrel resistance could be provided in the future by newer blockers of the $P2Y_{12}$ receptor, which are already under investigation in phase 3 clinical studies.[71-73] Finally, it is likely that in the future laboratory tests of platelet function will be routine examinations in patients with cardiovascular diseases. The clinical significance of performing such tests in patients who are being treated with antiplatelet agents in clinical practice must be determined by the results of large studies that will investigate the relation between inadequate inhibition of platelet activation and the risk of thrombotic cardiovascular episodes.

Conclusions

Given that today there is no generally accepted quantitative *ex vivo* method for the estimation of platelet activation after the administration of aspirin or clopidogrel, it is not possible to draw up specific guidelines for the treatment of patients who have elevated levels of platelet activation despite treatment with these drugs. Larger doses of aspirin (such as 325 mg daily) or complete patient compliance with therapy are probably sufficient measures for managing aspirin resistance in individual patients. In addition, in patients who do not respond to clopidogrel treatment, the administration of 150 mg per day as a maintenance dose should be considered as an alternative solution. In the near future, it may be possible to manage clopidogrel resistance with the use of new platelet $P2Y_{12}$ receptor blockers.

If laboratory tests of platelet function become routine in patients with cardiovascular disease, large studies will be needed to investigate their clinical significance in patients who are under antiplatelet treatment.

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