Mechanisms of Platelet Activation and Modification of Response to Antiplatelet Agents

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Key words: Platelets, antiplatelet medication, aspirin, clopidogrel, acute coronary syndrome.

Atherothrombosis is an evolving, multifocal and diffuse pathological process affecting the arterial wall, characterised by the development of atheromatous plaques and eventually their rupture and the subsequent activation of the coagulation cascade, leading to thrombosis. Increasing size and degenerative changes within the atheroma, such as ulceration, plaque rupture and intraplaque haemorrhage, initiate the thrombotic process, which is the main complication of atherosclerosis and results in the presentation of the acute coronary syndromes (ACS).¹⁻³

In the pathogenesis of atherothrombosis the following conditions play an important role:
1. Endothelium function impairment (endothelial dysfunction).⁴
2. Disturbances in the metabolism of lipids and lipoproteins.²,³,⁵
3. Chronic inflammation, which, via certain cytokines, chemokines, adhesion molecules and growth factors, modifies the function of the arterial wall cells, modulates their complicated cell-to-cell interactions, and promotes both the development and rupture of the plaque.⁶
4. Oxidative stress, which changes the structure and function of lipoprotein particles, causes cell activation, induces inflammatory processes in the arterial wall and promotes apoptosis.⁷
5. Hypercoagulable state, to which platelets, leucocytes, coagulation factors and inhibitors significantly contribute.³,⁸

Specifically, platelets play key roles in both the formation of the atheromatous plaque and acute thrombosis following plaque rupture, leading to the clinical presentation of acute atherothrombotic events.²,³,⁹ Notably, these patients may carry the atherosclerotic lesions for many years, but they exhibit significant symptoms and sometimes die because of the thrombosis. Thus, the therapeutic approach to these individuals aims at three major points:
1. Modification of the risk factors that

² This text derives from a meeting of the specialists mentioned in the author list and scientific committee members list, which was organised by the Hellenic Cardiovascular Research Society and held in Athens on March 18, 2009. This publication is supported by the Hellenic Cardiovascular Research Society and it summarises the conclusions of this meeting.
promote the development and progression of atherosclerosis, namely hypertension, diabetes mellitus and dyslipidaemia. Lifestyle changes (smoking cessation, weight loss, regular physical activity) are also essential.

2. Restoration of the occluded vessel’s patency, which is achieved by thrombolysis or percutaneous coronary intervention (PCI).

3. Inhibition of thrombus formation and expansion using antithrombotic agents (antiplatelets and anticoagulants).

Antithrombotic treatment is only one of the three therapeutic components; its role, however, is crucial because it prevents thrombosis, which leads to the clinical symptoms and cardiovascular death. Its role is important in primary prevention, reducing the possibility of thrombosis in patients with atherosclerotic disease, and also in secondary prevention, decreasing recurrent thrombosis in persons with previous thrombotic events and restenosis or de novo thrombosis in cases of PCI or bypass operations.

The present article interprets the scientific conversation between Greek scientists specialised in the field of atherothrombosis, during a special meeting organised by the Hellenic Cardiovascular Research Society and held in Athens in March 2009. The meeting focused on the specific role of antiplatelet therapy in patients with atherothrombotic disease, the possible adverse reactions and their treatment, as well as the potential ways of improving the efficacy of antiplatelet therapy.

**Platelets in the pathogenesis of atherothrombosis**

The complicated pathophysiological procedure underlying acute thrombotic episodes includes interactions between platelets, plaque components and coagulation factors. The rupture of the intima of a coronary artery, following a plaque rupture or a iatrogenic injury during PCI, exposes the sub-endothelial elements, such as von Willebrand factor and collagen, to the bloodstream. Specific receptors on the surface of the platelets (GPIa/IIa, GPIb/V/IX, GPVI, etc.) bind the former molecules, causing the platelet to adhere to the site of the endothelial injury.

Platelet adhesion leads to activation of the cell through intracellular metabolic cascades. As a result, platelets aggregate through fibrinogen bridges, which bind to the activated platelet receptor-integrin $\alpha$IIb$\beta$3 (GPIIb/IIIa). Activated platelets release biologically active substances, stored inside the cell or newly synthesised, among them adenosine diphosphate (ADP), arachidonic acid, platelet-activating factor (PAF) and serotonin, which induce and preserve platelet activation and aggregation through positive feedback mechanisms.

The secretion of pre-coagulant factors from the platelets (e.g. Factor V) and the interaction with the negatively charged phospholipids of the platelet membrane maximise the reaction of thrombin synthesis, which has been initiated by the intravascular exposure of tissue factor and is one of the most powerful platelet activators. These mechanisms may partly explain the recurrence of thrombotic episodes in patients already on antiplatelet medication, and justify the need for this type of drug in cases of acute ischaemic events.

ADP has a pivotal role in the platelet activation and aggregation process, acting on the purinergic receptors P2X1, P2Y1 and P2Y12 located on the platelet surface. These receptors act synergistically in the activation of the cell. The P2X1 receptor participates in the change of the platelet’s shape, while P2Y1 is responsible for the initial reversible activation and P2Y12 for the prolonged activation and aggregation of platelets.

Platelet activation also induces the release of arachidonic acid from the phospholipids of the cell membrane through the action of a cytoplasmic phospholipase $\text{A}_2$. Arachidonic acid is metabolised by cyclooxygenase (COX) -1, mainly towards prostaglandins G2/H2, and subsequently thromboxane $\text{A}_2$ (TXA$_2$) is formed by TXA$_2$ synthetase. This molecule further activates the platelets, acting on a specific surface receptor while exerting a pro-inflammatory effect on the cells of the arterial wall.

Apart from the established importance of platelet actions in the thrombotic procedure, they play a significant role in the formation of the atheromatous plaque. According to recent reports, they adhere to the endothelium under mild inflammatory conditions and attract monocytes, which penetrate the sub-endothelium and are transformed into macrophages and foam cells. Several adhesion molecules (P-selectin, ICAM-1) and chemokines (MCP-1, SDF-1, IL$\text{I}_{1}\beta$, IL-8, CD40L, RANTES, ENA-78, etc.) participate in these intercellular interactions and enhance the inflammation in the arterial wall. Apart from monocytes, endothelial progenitor cells (EPCs) are also recruited by the activated platelets and have the potential to transform into either foam cells, promoting atherogenesis, or into endothelial cells, leading to endothelial regeneration. The specific conditions that influence this procedure need to be elucidated further.
Antiplatelet medication

As previously mentioned, platelet activation and aggregation are pivotal steps in the formation and expansion of atheromatous lesions, the generation of thrombus after plaque rupture, and the synthesis of thrombin, which stabilizes the thrombus.

The inhibition of platelet activation and aggregation modifies these cardinal procedures. The clinical efficacy and safety of antiplatelet regimens consisting of aspirin and thienopyridines (mostly clopidogrel) was proven by:

1. A meta-analysis of 287 clinical trials regarding the secondary prevention of ischaemic cerebrovascular events in patients with atherothrombosis, performed by the Antithrombotic Trialists’ Collaboration, which showed that 75-150 mg aspirin per day reduces the incidence of ischaemic episodes by 25% compared to no antiplatelet therapy.

2. The CAPRIE study, which included 19,185 patients with atherothrombotic disease (recent cerebrovascular event, or acute myocardial infarction or symptomatic peripheral arterial disease) and compared the efficacy and safety of long-term clopidogrel (75 mg/day) with aspirin (325 mg/day) in the prevention of new atherothrombotic complication or vascular death. The risk of a new event per year was 5.32% for the clopidogrel and 5.38% for the aspirin group (p=0.043). The use of clopidogrel decreased the risk of new episodes by 8.7% compared to aspirin, without increasing the incidence of serious bleeding.

Aspirin and clopidogrel represent the cornerstone of modern orally administered antiplatelet medication, given the established reduction by nearly 25% of mortality and morbidity in patients with atherothrombosis. However, 10-20% of these patients suffer thrombotic complications even though they receive antiplatelet drugs. This percentage has been slightly reduced with the introduction of prasugrel, a novel third-generation thienopyridine.

ADP is a more potent platelet activator than arachidonic acid. Thus, pharmacologically speaking, clopidogrel has to be more powerful than aspirin. The combination of both drugs produces even better platelet inhibition (synergistic action). Prasugrel is more effective than clopidogrel, mainly because of its different pharmacokinetic profile, with rapid metabolic activation by CYP450 in the liver and fast achievement of high concentrations of the active metabolite in comparison with clopidogrel. The TRITON-TIMI 38 trial reported that prasugrel administration in patients with ACS undergoing PCI correlates with fewer major cardiovascular complications compared to clopidogrel, especially during the first month. Nevertheless, this agent is related with a significant greater risk of major haemorrhage, occurring mostly within the first month after the procedure.

As a result, the efficacy and safety of the antiplatelet medication can be further improved. This can be achieved in the middle term by the better use of existing drugs, based on a knowledge of pharmacokinetics, pharmacodynamics and pharmacogenomics. Novel substances are already in development (reversible P2Y12 antagonists such as ticagrelor and elinogrel, thrombin receptor PAR-1 antagonists) and they are expected, on a long-term basis, to reduce the frequency of major cardiovascular complications in patients with atherothrombosis.

Pharmacokinetic and pharmacodynamic properties of aspirin

Aspirin (acetylsalicylic acid) is rapidly and almost entirely absorbed by the stomach and duodenum. After administration of an oral dose of non-intestinal-soluble aspirin preparation, 80-100% of the drug is absorbed in 20 minutes to 2 hours. The maximum serum concentration is achieved in 30 min. In contrast, intestine-soluble tablets have a markedly reduced absorption, resulting in a bioavailability decreased by 40-50% for more than 3-8 hours after administration. The drug’s half-life in blood is low (15-30 min) owing to fast hydrolytic degradation by esterases of the intestinal mucosa, liver and blood. Thus, aspirin probably exerts its antiplatelet action mainly on the platelets of the portal circulation. The recommended daily dosage in patients with atherothrombosis (75-325 mg) is 3-10 fold higher than the minimum dose required to produce full inhibition of COX-1 (20-30 mg). This difference explains the very small inter-individual variability in terms of response to the drug. Aspirin’s activity is not influenced by gender, age or platelet count. The most important mechanism of aspirin’s antiplatelet action, in doses used in cardiovascular disease, is the irreversible acetylation of the –OH moiety of serine-529 in the active centre of COX-1. This chemical modification inhibits the ligation of arachidonic acid inside the active centre, a necessary procedure for TXA2 synthesis. A reduction in TXA2 synthesis of at least 90% is required to obtain effi-
cient inhibition of platelet aggregation. In most patients this is accomplished with 30 mg/day, and the antiplatelet result remains virtually the same if the dose exceeds 80-100 mg/day. In high doses, COX-2 is also blocked and the anti-inflammatory action becomes apparent. Moreover, in high concentrations, aspirin inhibits COX-1 and, to a lesser degree, COX-2 in endothelial cells, reducing the synthesis of PGI2 (prostacycline), which has a significant antiplatelet action.28

Aspirin is a rather weak inhibitor of platelet activation induced by thrombin, ADP, serotonin or adrenaline. COX-1 in platelets exposed to aspirin is inactivated permanently, for the rest of the cells’ life—a period that usually reaches 7-10 days, since platelets as terminally differentiated cells have lost their nuclei and are incapable of synthesising new molecules of the enzyme. As a result, the restoration of platelet activity depends on the production of new platelets from the bone marrow. Notably, as nearly 10% of the circulating platelets are replenished every day, almost 30% of platelets will have fully active COX-1 and normal production of TXA2 48 hours after the last aspirin dose. Apparently, daily aspirin administration is recommended over every second day regimen.

**Pharmacokinetic and pharmacodynamic properties of clopidogrel**

Clopidogrel is a member of the thienopyridine family, along with ticlopidine and prasugrel, and is a powerful antiplatelet agent.28 It is a prodrug, which is absorbed in the gut with the aid of the ABCB1/MDR1 protein transporter. Subsequently, it is converted to the active metabolite by several isoforms of cytochrome P450 in the liver, mainly CYP2C19, CYP3A4, CYP3A5, CYP1A2, CYP2B6 and CYP2C9 also participate in the procedure. The maximum concentration of the active metabolite in blood is reached within 1 hour after the administration of 600 mg clopidogrel.29 Interestingly, 85% of the absorbed drug is hydrolysed by plasma and intestinal mucosa esterases to form inactive products. The half-life of the active metabolite after a single or multiple doses is about 8 hours.30 This active metabolite is a potent selective inhibitor of the P2Y12 ADP receptor, which exerts its action by forming disulphuric bonds with two serine residues (ser-17, ser-270) of the receptor molecule. This chemical modification causes an irreversible inhibition of ADP ligation to the receptor that in turn leads to an increase in levels of cyclic adenosine monophosphate (cAMP) in the cytoplasm of the platelet. The resulting phosphorylation of vasodilator-stimulated phosphoprotein (VASP) finally inhibits the activation of the GPIIb/IIIa receptors and eventually platelet aggregation. The maximum inhibitory activity of clopidogrel is reached in 24 hours after administration of 75 mg, in six hours after 300 mg, and in two hours after 600 mg. As the P2Y12 receptor blockade is irreversible, and 10% of platelets are renewed daily, at 5 days after treatment cessation 50% of the circulating platelets will be completely functional and capable of producing adequate hemostasis.

The final outcome of clopidogrel use, namely the inhibition of platelet activation by ADP, is influenced by several factors. The absorption (intestinal mucosa) and metabolism of the drug (esterases, CYP450 isoforms) consist of a sequence of events that define the quantity of the active drug produced.31 There is also a close relationship between its plasma concentration and the rapidity and level of inhibition of platelet activation. Furthermore, conditions affecting the affinity of clopidogrel with the P2Y12 receptor can also influence its action.

The significance of the former remarks regarding the factors that can alter clopidogrel's effectiveness is becoming apparent nowadays, as numerous generic forms of clopidogrel are widely available. They differ from the prototype drug (clopidogrel bisulphate), only in the chemical composition of the clopidogrel salt, which can be either besylate or hydrochloride. The health authorities of the European Union have approved the generic products using different salts but they are not marketed in the USA. Studies performed on healthy volunteers have established the bio-equivalence between the generic clopidogrel salts and the prototype drug. However, pharmacokinetic and pharmacodynamic data regarding this equivalence in the setting of ACS and in patients undergoing PCI are still lacking.32 This cognitive defect has triggered an extensive debate among specialists, and many national cardiovascular societies have proposed avoiding the use of generic forms in ACS and in cases of PCI.33 The Greek National Organisation for Medicines and the European authorities have endorsed this position. Thus, so far, the generic forms of clopidogrel are indicated for the secondary prevention of atherothrombosis, in patients with previous myocardial infarction, stroke or established peripheral arterial disease. It becomes obvious that these agents are still well differentiated in terms of clinical indications from the prototype clopidogrel bisulphate, which, apart from secondary prevention, is considered necessary in the acute phase of thrombotic episodes (ACS), during
PCI and for the following year, and is indicated for patients suffering from diseases derived from the whole spectrum of atherothrombosis.

Pharmacological interactions and response modification in antiplatelet treatment

Aspirin and clopidogrel present major interactions with 58 and 50 known drugs, respectively. Knowledge of the ones that can potentially reduce the antiplatelet efficacy of aspirin or clopidogrel and affect clinical outcomes is crucial. The most significant interactions concern those with:

1. drugs used in the treatment of atherothrombosis (statins, etc.);
2. drugs used to ameliorate the gastrointestinal side effects of aspirin (protein pump inhibitors);
3. drugs used in the therapy of other concomitant diseases (non-steroidal anti-inflammatory drugs, etc.)

Antiplatelet medication and statins

Aspirin and statins

There is evidence that co-administration of aspirin with statins has a synergistic action in the secondary prevention of atherothrombosis. A recent study has shown that long-term treatment with both drugs in patients with ACS decreases the relative risk of a recurrence by 24% compared with aspirin monotherapy. This observation needs confirmation in studies with greater numbers of patients.

Clopidogrel and statins

After the study by Lau et al was published, an intense debate took place addressing the issue of clopidogrel action inhibition by simultaneous administration of lipophilic statins metabolised by CYP450 3A4. For the time being, on the basis of the results of the study by Mitsios et al and other supporting studies, the scientific community accepts that lipophilic statins such as atorvastatin, rosuvastatin, etc., do not influence the antiplatelet, efficacy of clopidogrel.

Interactions with non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are widely used and, apart from their adverse gastrointestinal effects, they interfere with the metabolic pathway of arachidonic acid, causing temporary inhibition of platelet activation. More specifically, agents like ibuprofen and indomethacin produce reversible inhibition of COX-1 through acetylation at the same enzyme site as aspirin. Ibuprofen administration before aspirin antagonises aspirin’s binding to COX-1. Since the half-life of aspirin is only 15-20 min, the drug has been cleared from the blood by the time COX-1 is again vulnerable to inhibition and so no antiplatelet action is achieved. In a retrospective study in patients receiving aspirin after hospital discharge, the subjects on concomitant ibuprofen use faced a twofold increased risk of death compared to those taking aspirin alone, or with other NSAIDs such as acetaminophen. It is assumed that for every 1000 patients on aspirin treatment, 12 deaths per year can be attributed to NSAID use. Clopidogrel has not been reported to interact with NSAIDs so far.

Antiplatelets and protein pump inhibitors (PPIs)

As previously mentioned, co-administration of clopidogrel with drugs metabolised by or interacting with CYP450 isoforms, and especially CYP2C19, may result in reduced production of the active metabolite of the prodrug and subsequently the loss of its antiplatelet potency when used in a maintenance dose (75 mg). Aspirin has no hepatic metabolism and is free of such effects. Clopidogrel’s interaction with PPIs deserves special mention because these drugs are widely used to prevent and treat the gastrointestinal adverse effects related to antiplatelet medication.

PPIs are metabolised in the liver by several CYP450 isoforms; thus, they can interact with thienopyridines, reducing their active metabolite concentrations, their antiplatelet efficiency, and exposing patients to increased risk for cardiovascular complications. This hypothesis was tested in a series of clinical and laboratory studies characterised by great heterogeneity in terms of study design and conclusive results. We intend to present the most important, along with their meta-analysis.

In 18,565 patients receiving clopidogrel after an ACS or PCI, the incidence of new myocardial infarction was 2.6% in the subgroup taking PPI and 2.1% in the other patients. Mortality was 1.22% and 1.2%, respectively. The results of PRINCIPLE-TIMI 44, which assessed the inhibition of platelet aggregation 6 h after the administration of clopidogrel 600 mg or prasugrel, and TRITON-TIMI 38, which compared
the efficacy and safety of prasugrel with clopidogrel over a composite endpoint of mortality and incidence of myocardial infarction (MI) or stroke, were recently subjected to post hoc analysis. In the first study, in subjects receiving clopidogrel and PPIs platelet aggregation was 35% greater than in those receiving clopidogrel alone. In prasugrel recipients, PPI administration increased platelet aggregation by 10%. However, subgroup analysis of TRITON-TIMI 38 did not show any significant correlation between PPI administration and mortality or the occurrence of major cardiovascular events.44

Another study, involving 1425 patients with ACS undergoing PCI who received a 600 mg loading dose of clopidogrel, reported that PPI use for at least one week prior to the intervention was related with a 20% increase in platelet aggregation.45

Recently a meta-analysis of eight clinical and laboratory trials was published, evaluating the effect of co-administration of clopidogrel and PPIs on the morbidity and mortality of patients with atherothrombosis.46 Two studies showed that PPIs negatively affected clopidogrel’s action with respect to platelet aggregation, but two others did not confirm this finding. Three out of four clinical trials analysed reported a significant increase in MI or stroke occurrence, hospitalisation due to cardiovascular reasons, or death, when PPIs were use concomitantly with clopidogrel and aspirin.

Another recent meta-analysis focused on the mortality and frequency of major cardiovascular events. A total of 93,278 patients from 23 trials were analysed. Despite some heterogeneity among the trials, no significant increase in cardiovascular risk or mortality was attributed to PPI usage.47 So far, there are no prospective randomised trials addressing this issue and some small studies have come up with conflicting results.

In one small trial, 104 patients undergoing PCI and receiving aspirin 75 mg and clopidogrel 150 mg maintenance dose for 30 days were randomised to either omeprazole or pantoprazole.48 The incidence of reduced clopidogrel response (evaluation by VASP method) was two times greater in the omeprazole group. In contrast, in a recent small prospective observational study omeprazole did not alter the incidence of major cardiovascular events in PCI patients.49

Clopidogrel-PPI interaction was also assessed in a large retrospective case-control study analysing 13,636 individuals who were admitted due to myocardial infarction and were discharged on clopidogrel treatment. Of these, 734 were rehospitalised because of a new MI within 90 days. Multivariate analysis showed that, compared to a control group (n=2057), concomitant use of PPIs was related with an increase in the relative risk for new thrombotic episode by 1.27% (95% CI: 1.03-1.57) only for the first 90 days after the first admission. Classification by PPI type showed that pantoprazole, which does not inhibit CYP450 2C19, did not correlate with the increase in thrombotic events.50 On the basis of available evidence, the US Food and Drug Administration (FDA) suggests that patients on clopidogrel therapy should consult their doctor in order to initiate or withdraw PPI treatment.51 According to the FDA, co-administration of clopidogrel and omeprazole causes a 45% and 47% reduction in the concentration of the active clopidogrel metabolite and in platelet inhibition, respectively. Omeprazole inhibits clopidogrel equally, regardless of the administration timing, as long as it is given within 12 hours after clopidogrel. Esomaprazole exerts an action on CYP2C19 comparable to omeprazole and the FDA suggests avoiding co-administration of omeprazole or esomaprazole with clopidogrel, noting that we lack data regarding the interaction of other PPIs with clopidogrel. Nevertheless, it advises doctors to be very careful when prescribing PPIs in general to patients who are already on clopidogrel. The recent report from the Committee for Medicinal Products for Human Use of the European Medicines Agency states, in accordance with the FDA’s suggestions, that the simultaneous use of clopidogrel with omeprazole or esomeprazole, but not other PPIs, should be discouraged.

The FDA also recommends avoiding administering other drugs that inhibit CYP2C19, such as cimetidine, fluconazole, voriconazole, etravirin, felbamate, fluoxetine and fluoxetine, together with clopidogrel. As far as other gastroprotective agents are concerned—namely ranitidine, famotidine or nizatidine—there is no evidence to show that they can affect clopidogrel’s efficacy.52 It should be emphasised that PPIs are the established therapeutic strategy of choice for patients receiving antiplatelet agents who are at risk of gastrointestinal bleeding. Furthermore, the trials evaluating PPIs’ influence on clopidogrel’s action are prospective observational studies. So far, no double-blind randomised trials have examined this issue.

**Resistance to antiplatelet treatment**

Using oral antiplatelet drugs in secondary prevention reduces the risk of MI and cardiovascular death
by 25% and 20%, respectively. However, 15% of patients with coronary artery disease who are under continuous antiplatelet therapy will need additional admission due to a new MI or stroke. This observation points up the need for the improvement of the efficacy of antiplatelet therapy and raises the issue of early recognition of antiplatelet drug resistance. This term encompasses two entities:

1. Clinical resistance. This refers to patients who suffer a new thrombotic episode while receiving antiplatelet medication. We should point out that thrombosis is a complex procedure and platelet activation is only one step of it. Moreover, each antiplatelet agent acts on one of the several metabolic pathways underlying platelet activation. Thus, the nature and intensity of the thrombogenic insult is important in overcoming the antiplatelet effect of a single substance. Another factor that has to be kept in mind is the possibility of wrong estimation of the cause of thrombosis, which results in the use of improper medication.

2. Laboratory resistance. This is defined as the failure of antiplatelet therapy to inhibit platelet function, as evaluated \textit{ex vivo} by one or more acceptable laboratory methods. A significant number of clinical and laboratory studies are now in progress, aiming to predict which of the patients with established laboratory resistance to antiplatelets are at risk for clinically overt resistance.

The major pathophysiological mechanisms responsible for resistance to aspirin and clopidogrel are depicted in Table 1.

### Laboratory methods used to evaluate antiplatelet drug resistance

These methods are divided into three categories.

#### Specialised biochemical methods

These allow the measurement of certain metabolites in plasma or urine whose concentration changes according to the activation state of platelets, as well as the concentration of the active metabolites of clopidogrel and prasugrel in plasma.

- **Aspirin resistance** can be evaluated directly by measuring TXA\(_2\) production, which increases during platelet activation and is inhibited by aspirin. TXA\(_2\) is an unstable molecule and is metabolised rapidly towards more stable forms like TXB\(_2\), which can be measured in the serum, and 11-dihydroTXB\(_2\), which is found in the urine. The levels of these metabolites are dependent upon COX-1 activity; thus, their measurement is a reliable index of the efficacy of small aspirin doses.

- **Thienopyridine resistance.** During clopidogrel or prasugrel therapy, two parameters may feasibly be measured:
  i. Active metabolite levels;
  ii. The phosphorylation of phosphoprotein VASP inside the platelet cytoplasm. A specific monoclonal antibody labelled with a fluorescent agent is used and the measure is achieved with flow cytometry.

This facility helps us evaluate platelet response to thienopyridine therapy, which is quantified using the Platelet Reactivity Index (PRI).

#### Functional methods

These can evaluate the effect of antiplatelet therapy on platelet activation induced by several agonists. The common feature of all these methods is that they use different platelet activators (ADP, collagen, arachidonic acid) sequentially and using the blood sample from a single puncture, and thus they make it possible to check the antiplatelet action of different drugs (aspirin, clopidogrel, prasugrel, Ib/IIIa inhibitors). With these methods we can determine platelet reactivity to
Resistance to Antiplatelet Medication

arachidonic acid (aspirin therapy) or ADP (clopidogrel or prasugrel therapy), simulating the normal cell environment with the presence of plasma and blood cells, and we can obtain more information compared to the biochemical methods described previously.

Each of the available applications has a different functional mechanism and evaluates a different aspect of platelet reactivity. In this way, the functional methods are considered complementary to each other, which is why none of them is indicated for routine clinical use. According to recent data and experience from specialized centres, the simultaneous use of different functional methods leads to a more accurate approach to the complicated biochemical procedure of platelet activation and hypercoagulable state.

The most thoroughly evaluated and verified functional methods are the following:

- **Light transmittance aggregometry** is the most widely used and is considered the method of choice. It requires the use of platelet-rich plasma (PRP) and is based on the alteration of the optical density of the platelet solution during activation by ADP, arachidonic acid, collagen, adrenaline or thrombin receptor activating peptide (TRAP). It provides us with ample information but it is time consuming and technically demanding, especially with regard to sample preparation, and it is rather difficult to apply in daily practice.

- **Impedance aggregometry** is performed in whole blood specimens and determines the adhesion and aggregation of platelets. The primarily measured parameter is the change in electrical impedance occurring when platelets are activated by natural activators (ADP, arachidonic acid or TRAP), and adhere and aggregate upon two electrodes placed in the sample. Today this technique is used as a point-of-care method using the “Multiplate” (Dynabate) analyser, which has been used in several clinical trials in patients with coronary heart disease, with reliable results, and does not require sophisticated sample preparation since the measurement is performed in whole blood.

- **Whole blood aggregometry using VerifyNow (Accumetrics)**. This method is also performed in whole blood and evaluates platelet aggregation under the influence of natural activators. The determination is based on the optical density changes. It is quite easily done and no special sample preparation is needed. It can be used as a point-of-care method and its clinical value has been examined thoroughly in patients with coronary heart disease. Several trials, such as the GRAVITAS trial, are in progress to evaluate VerifyNow as a point-of-care technique.

- **Platelet function analysis with the PFA-100 system (Siemens)**. This is a method that measures the time needed for the blood flow to be disrupted in a small tubule whose walls are covered in platelet activators. It uses whole blood samples and until recently only kits for determining platelet response to aspirin were available. Today, thienopyridine resistance can also be measured and the method is under evaluation as a point-of-cares technique by clinical trials in progress.

- **Thromboelastography-platelet mapping** uses whole blood and allows for the evaluation of the contribution of platelet activation to the kinetics of thrombus formation and the quality characteristics of the thrombus itself. It is currently being tested in patients with acute coronary syndromes.

**Pharmacogenomic methods**

The presence of single nucleotide polymorphisms (SNPs) in the genes encoding either for the transfer protein ABCB1, responsible for thienopyridine absorption from the intestinal mucosa, or different isoforms of CYP450, like CYP2C19, which control thienopyridine metabolism in the liver and production of their active metabolites, results in significant modification of these agents’ pharmacokinetic and pharmacodynamic properties. Recent prospective trials showed that, in patients receiving clopidogrel post PCI, the presence of SNPs in these genes correlates with a significant increase, up to fourfold, of the risk for major cardiovascular events during the first 12 months.53

It should be underlined that all the previously mentioned biochemical, functional and pharmacogenomics methods require the presence of specialised personnel. Platelets are very sensitive, and their activation depends on the anticoagulants used, the venous puncture (blood collection through a venous catheter and disposing of the first 2 ml of blood is required), the measurement timing, the agonist concentration, etc. These difficulties are partly ameliorated when point-of-care methods are used.

**Clinical consequences of laboratory antiplatelet resistance**

**Aspirin resistance**

The platelet response to aspirin, as determined by biochemical or functional methods, is an all-or-none
phenomenon. The prevalence of aspirin resistance differs with respect to the evaluation method used but it reaches 5-9% in patients with coronary heart disease who receive the drug. Recent studies report this percentage to be 40% lower in individuals who are on a double antiplatelet regimen.

Clopidogrel resistance

This is related to the degree of in vitro inhibition of ADP-induced platelet activity in patients taking clopidogrel. In this population, this parameter presents a normal distribution curve (Gaussian distribution). At the margins of the curve, 4% do not respond to the drug at all and another 4% present full inhibition of platelets. For this reason the patients’ response to clopidogrel is described as ‘variable’. Among the common reasons for resistance to clopidogrel, the patient’s non-compliance holds an important position. Resistance to aspirin and —mainly— clopidogrel changes over time in nearly 30% of patients and is affected by the presence of inflammation or a hypercoagulable state. Often, resistance to clopidogrel coexists with cardiovascular risk factors (hypertension, dyslipidaemia, diabetes mellitus), although it is unclear whether modification of the risk factors alters the antiplatelet resistance status. Moreover, platelet response to clopidogrel seems to differ according to the salt used in the drug preparation, as a recent study reported after administering 300 mg of prototype clopidogrel and benzyl-sulphuric clopidogrel salt sequentially to healthy volunteers.

An additional problem in diagnosing resistance to antiplatelet medication is the determination of the normal value range for every method, which is necessary to avoid both a pro-thrombotic condition and haemorrhagic complications. A series of prospective studies have defined the therapeutic range (optimal residual platelet activity) for Multiplate, VerifyNow and Whole Blood Aggregometry techniques. In any case, the genomic evaluation of a patient, combined with biochemical and functional methods, is expected to improve the efficacy of antiplatelet treatment.

Management of antiplatelet drug resistance

Platelet activation in patients with atherothrombosis is a complicated procedure. Successful inhibition of this phenomenon with antiplatelet medication is a therapeutic challenge with significant beneficial effects in terms of the patient’s survival. Aspirin and clopidogrel are effective agents with a large therapeutic window, which allows for dose adjustments without significant safety compromise. The antiplatelet medication available so far provides us with the following treatment options:

1. Loading therapy during PCI:
   a. Aspirin up to 325 mg and clopidogrel 300 mg, which is the suggested regimen for all patients undergoing PCI.
   b. Aspirin up to 325 mg and clopidogrel 600 mg. This is suggested in high-risk patients and is associated with less laboratory resistance to clopidogrel.
   c. Aspirin up to 325 mg and prasugrel 60 mg.

2. Maintenance therapy in patients with stent placement:
   a. Aspirin up to 325 mg/day and clopidogrel 75 mg/day is recommended for all patients.
   b. Aspirin up to 325 mg/day and clopidogrel 150 mg/day. This therapeutic option is being evaluated for high-risk individuals with resistance to clopidogrel.
   c. Aspirin up to 325 mg/day and prasugrel 10 mg/day. This scheme was tested in the TRITON-TIMI 38 trial and proved efficient, though causing a significant increase of bleeding risk.

In daily practice, physicians face the following issues regarding antiplatelet drug resistance:

1. Antiplatelet therapy modification in patients presenting with a thrombotic episode during single (aspirin or clopidogrel) or double (aspirin and clopidogrel or prasugrel) therapy.
In this category, antiplatelet resistance evaluation in specialised laboratories will suggest whether the episode is related to drug resistance and will guide appropriate therapy. This approach is recommended by the ESC and ACCP guidelines.

2. **Diagnosis of clopidogrel resistance within 48 hours from PCI and stenting and selection of the best antiplatelet regimen in order to avoid major cardiovascular complications.**

The clinical value of this approach has been established by large prospective trials, which tested the effectiveness of Multiplate, VerifyNow, aggregation in PRP and VASP measurement. These studies showed that within one month post PCI the risk for cardiovascular complications is increased by 5-8 times. However, given the special technical characteristics of the methods described previously, specialised medical teams should evaluate the results. The efficacy and safety of the available therapeutic options has not been examined in prospective randomised trials and thus no treatment modification can be recommended, unless it represents a decision of cooperating specialised clinical and laboratory scientific groups and is applied to high-risk patients. A repeated measurement of the response to the antiplatelet treatment is considered essential.

3. **Diagnosis of resistance to aspirin or clopidogrel in high-risk patients with remote ACS or PCI, who are receiving long-term antiplatelet therapy.**

These patients are of particular interest, because they sometimes suffer from additional comorbidities (cancer, inflammatory diseases) or undergo surgical operation, which can alter the response to antiplatelet therapy and increase cardiovascular risk. The number of trials addressing this clinical setting is minimal, although the optimisation of antiplatelet medication is of cardinal significance in such cases. In view of the lack of data, the laboratory control and decision making should be carried out by groups of specialists. Extreme precautions should be taken when a generic form substitutes for prototype clopidogrel. These patients must undergo an evaluation of clopidogrel response, because the change of the salt contained in the drug preparation might affect the level of platelet inhibition.79

As far as the genetic polymorphisms influencing the metabolism of clopidogrel are concerned, the FDA has recently published a report regarding the management of patients with decreased production of the active clopidogrel metabolite (poor metabolisers). This report underlines that doctors should be aware of the availability of genetic tests to diagnose CYP2C19 polymorphisms. It also advises physicians to use different doses of clopidogrel, or even different antiplatelet agents, in these patients.80

Apart from the abovementioned actions, one could hypothesise that the use of other antiplatelet agents may be an alternative in cases of documented resistance to the abovementioned agents. In this context triflusal has been approved for the prevention of thromboembolism in patients with atrial fibrillation and has also been tested in patients after acute MI.81 Finally, the development of novel antiplatelet drugs may provide a therapeutic option for these patients. In this regard, the results of the PLATO82 and TRITON25 trials are very promising and show that these new drugs may be useful in the treatment of patients with clinically established resistance to aspirin and/or clopidogrel.

**Conclusions**

The analysis presented here reflects a scientific debate that took place during the special meeting held in Athens on March 2009, under the auspices of the Hellenic Cardiovascular Research Society, and concisely summarises all the recent evidence regarding the activation of platelets, their role in atherothrombotic disease, the special issues of antiplatelet therapy, as well as the difficulties encountered and the management of adverse reactions during antiplatelet treatment in patients with acute coronary syndromes.

The main conclusions of this meeting were:

1. Platelets play a pivotal role in the pathogenesis of atherothrombosis.
2. Antithrombotic treatment is one of the three axes forming the global therapeutic approach to atherothrombosis, as it aims at the inhibition of thrombosis, which is the main clinical aspect of the disease and more importantly, the main cause of patient death.
3. Aspirin and clopidogrel are considered the cornerstone of modern oral antiplatelet medication, reducing the morbidity and mortality in atherothrombosis by 25% without significantly increasing the risk of major bleeding.
4. A thorough knowledge of the pharmacokinetic and pharmacodynamic characteristics of the antiplatelet agents commonly used by clinical cardiologists is essential for successful therapy.
5. The knowledge of basic interactions of antiplatelet drugs with other commonly used agents, concerning either atherothrombosis or other disease, is also crucial.

6. From 10-20% of patients suffer thrombotic events while on antiplatelet medication. Improvement of the treatment efficacy is a serious therapeutic challenge. Advanced medication requirements can be met either by the development and usage of new agents and drug combinations, or by an improvement in the use of current regimens. The adaptation of antiplatelet treatment intensity and duration according to the patient profile in an individualised manner is the target of modern antiplatelet therapy.

7. Aspirin or clopidogrel resistance in patients receiving double antiplatelet medication after PCI and stenting is a common feature and represents an independent risk factor for new major cardiovascular events.

8. Significant progress has been made in the development of laboratory methods to diagnose resistance to antiplatelet drugs. The currently available techniques are feasible and reliable. Moreover, the combined use of more than one of them allows for a global evaluation of the response to antiplatelet treatment.

9. Laboratory resistance to antiplatelet drugs increases fivefold the risk of major cardiovascular events within one month after PCI. The recognition of high-risk patients who do not respond well to clopidogrel and the appropriate adaptation of antiplatelet medication intensity can improve the clinical outcome significantly.

10. Given the lack of extensive clinical data from large trials and the methodological particularities of diagnostic facilities, specialised scientific teams should be responsible for the diagnostic evaluation and therapeutic approach to patients resistant to antiplatelet medication.

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