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EXPERT REVIEW

Old and New Molecular Mechanisms Associated with Platelet Resistance to Antithrombotics

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ABSTRACT Current available data show that about 5 to 40% of coronary patients treated with conventional doses of antithrombotic drugs do not display adequate antiplatelet response. Nowadays, aspirin remains the main antiplatelet therapy. However, a significant number of patients show platelet resistance to aspirin therapy, and recurrent thrombotic events occur. Combined antithrombotic therapies with thienopyridines, such as clopidogrel have been used to resolve this problem. However, clopidogrel treatment has been also associated with wide response variability, and non-responsiveness to clopidogrel also occurs in some patients. Therefore, the main question arising about the antithrombotic therapy is why particular patients do not benefit from the therapy and how they might be identified to improve their treatment. Different hypotheses have been suggested, including genetic factors, platelet heterogeneity, non-compliance and others. However, it is probably that many molecular mechanisms involved in platelet resistance to antithrombotic therapies still remains unknown. New technologies, such as proteomics and genetic, are beginning to show new unknown biological biomarkers and molecular mechanisms which may be associated with platelet antithrombotic drug resistance.

KEYWORDS antiplatelet agents · aspirin resistance · proteomics

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ANTIPLATELET TREATMENT WITH ASPIRIN

Advances in the understanding of the central role of platelets in the pathophysiology of cardiovascular disease have increased the investigations into the mechanisms of action of antiplatelet agents. Antiplatelet drug therapy is a cornerstone treatment for patients with cardiovascular diseases. Two of the main used antiplatelet drugs are aspirin and clopidogrel (P2Y12 receptor antagonists). Both of these drugs have demonstrated clinical beneficial effects in cardiovascular patients. However, it is known that a number of patients submitted to antiplatelet drugs do not respond to them since these patients have recurrent cardiovascular events. This suggests the existence of non-responsive patients to antiplatelet therapy. Different reasons and mechanisms for non-responsiveness to antiplatelet treatment have been discussed. This review focuses on the main mechanisms that try to explain non-responsiveness to both aspirin and ADP P2Y12 receptor antagonists.

ASPIRIN

The use of aspirin can be traced back to ancient Greece in 460 B.C. when Hippocrates, considered as the father of modern medicine, used the bark and leaves of *Salix alba* (willow) for the treatment of general pain and fever. There was probably controversy surrounding the use of aspirin in ancient Greece, since Dioscorides preferred coriander and not willow bark for pain. We can not know whether patients from Dioscorides were merely resistant to the beneficial properties of aspirin because thousands of years later we are still trying to understand it.

As mentioned, the use of willow bark was widespread in ancient times; for example, papyri of ancient Egypt

recommended the use of infusions of willow leaves to treat rheumatic pains. However, it was not until 1828 that Dr. Johan Buchner isolated a small quantity of a yellow powder extracted from willow bark, which he called salicilin and that appeared to be responsible for the beneficial effects of this plant. Later, Raffaelle Piria obtained pure salicylic acid and in 1853 the chemist Charles Gerhardt obtained the acetylsalicylic acid formula. However, it was in 1897 when Felix Hoffmann rediscovered Gerhardt's formula and developed the formula of aspirin marketed by Bayer Company.

The name *aspirin* was given to the new drug by Bayer's chief pharmacologist, Heinrich Dreser, who was anxious to find a name that could not possibly be confused with salicylic acid. At least two accounts are given for Dreser's choice of name: some authorities maintain that the drug was named after St. Aspirinius, an early Napolitan bishop who was the patron saint against headaches; a more prosaic explanation is that the name comes from the genus of plants to which meadowsweet belongs. According to this latter explanation, the acid derived from *spirarea* became *spirsaüre* in German. Acetylation of *spirsaüre* produced *acetylspisaüre*, which was soon shortened to *aspirin*.

At the beginning, aspirin was used only as an analgesic and antipyretic drug. The antithrombotic properties of aspirin were reported for the first time in the *Mississippi Valley Medical Journal* in 1953.

The efficacy of aspirin in the treatment and prevention of cardiovascular disease is well established (1,2). Multiple studies have demonstrated that aspirin is effective for both primary and secondary prevention, reducing by 25% the risk of major cardiovascular events (3–5). However, the mechanisms for the antiplatelet effect of aspirin are not clear at all. The main mechanism of aspirin's antiplatelet action was identified by the British pharmacologist John Vane, who described that aspirin inhibits the enzyme cycloxygenase (COX), thereby preventing the production of prostaglandins and, particularly in platelets, inhibiting thromboxane A_2 (TxA_2) synthesis by at least 90%. However, the clinical benefit showed by aspirin seems to be overcome by the inhibition of TxA_2 production by platelets. Therefore, other mechanisms for platelet inhibition were proposed, and they may contribute to the clinical benefits of aspirin. In this sense, aspirin facilitates the inhibition of platelet activation by neutrophils, an effect mediated by nitric oxide (NO)/cylic GMP (cGMP)-dependent process (6) (Fig. 1). Moreover, the endothelium may also release NO by aspirin, also inhibiting platelet activation (7). Aspirin may help to decrease the progression of atherosclerosis by protecting LDL from oxidative modification and also improves endothelial dysfunction in atherosclerosic vessels, two actions that also reduce platelet activity (8,9) (Fig. 1). Moreover, Santos *et al.* found that red

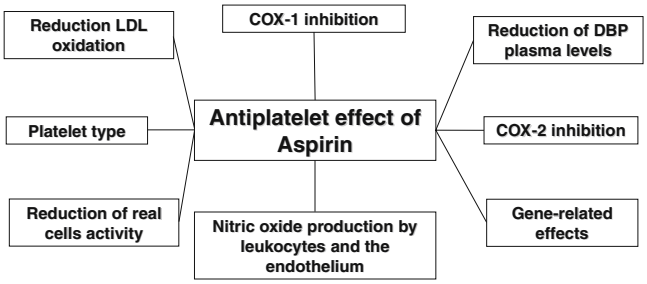

Fig. 1 Different mechanisms associated with the platelet response to aspirin. Abbreviations: DBP: vitamin D binding protein.

blood cells mediated platelet activation was reduced by aspirin (10) (Fig. 1). However, as mentioned above, aspirin does not inhibit platelet function as expected in a substantial proportion of patients. In this regard, despite of the clearly clinical benefits of aspirin, the potential impact of aspirin resistance is important because its prevalence has been estimated to be between 5% and 45% of the population. Particularly it is relevant in the diabetic population in who there is convincing data in the literature to suggest inadequate cardiovascular protection by aspirin. As example, in a meta-analysis of 287 randomised trials, antiplatelet treatment (aspirin in most studies) reduced the risk of ischemic events by 22%, but the risk reduction in the subgroup with diabetes was only 7% (11).

HOW ASPIRIN RESISTANCE MAY BE IDENTIFIED

The term *aspirin resistance* has been used in a clinical and laboratory context. Clinical aspirin resistance refers to the inability of aspirin to protect individuals with cardiovascular thrombotic events, while laboratory aspirin resistance is the lack of effect of aspirin on its antiplatelet effect. There are several methods to measure platelet resistance to aspirin, and, indeed, there is a wide discussion about what is better to identify platelet resistance to aspirin. Laboratory methods include turbidimetric aggregometry, using platelet-rich plasma and whole blood platelet aggregometry, and the Platelet Function Analyzer (PFA-100) and Ultegra Rapid Platelet Function Analyzer tests (RPFA-100). Moreover, urinary thromboxane determination has been also used to identify patients with aspirin resistence. However, this latter method cannot distinguish the source of thromboxane.

In clinical practice, arachidonic acid-induced platelet aggregation has been considered the gold standard test for measuring the antiplatelet effect of aspirin and seems to be much more widely available than measures of thromboxane in the serum or urine (12). The semiautomated platelet

function assays, such as PFA-100 or RPFA, have appeared as simple and rapid tests whose results seem to better correlate with clinical events, and their use is gradually increasing (13). PFA-100 combines two agonists in cartridge from closure time and time required for platelets to effect full occlusion of an aperture into a membrane coated with the platelet agonists. However, it is controversial which one of the laboratory methods should be used to detect aspirin-resistant patients. It is probably due to the large variability in the results obtained between the different published studies. It could be influenced by the population studied, the laboratory test used to diagnose platelet resistance to aspirin and also the dose of aspirin of the studied patients (14,15). Studies are needed to find an easy and rapid laboratory test to identify patients who do not benefit from aspirin treatment.

CORRELATION BETWEEN CLINICAL OUTCOME AND ASPIRIN RESISTANCE

The clinical diagnosis of aspirin resistance can only be made in retrospect because it is based on the occurrence of atherothrombotic ischemic events in patients while taking aspirin. Therefore, from a clinical point of view, aspirin resistance may be defined as the resistance to thrombotic events while taking aspirin. One of the first studies that showed a clear association between platelet resistance to aspirin and the risk of serious vascular events was conducted by Grotemeyer *et al.* in 1993 (16). In this study, one-third of the studied patients with cerebrovascular disease were considered aspirin-non-responder patients, since in a two-year follow-up period, 40% of patients experienced a new serious cerebrovascular ischemic event (16). Most studies have been performed correlating the platelet resistance to aspirin using laboratory test and the clinical outcome of cardiovascular patients. A sub-study of the Heart Outcomes Prevention Evaluation (HOPE) showed a linear association between high urinary levels of the main metabolite of thromboxane A_2 (11-dehydrothromboxane B_2) and the incidence of suffering a cardiovascular event (17). This sub-study included 976 patients at high risk of vascular events who were taking aspirin doses between 75 and 325 mg/day at inclusion. In this study, Eikelboom *et al.* observed that patients in the upper quartile of urinary 11-dehydrothrombosone B_2 excretion levels had a 3–5-fold higher risk of cardiovascular death compared with those in the lowest quartile (17).

Gum and co-workers also found a significant correlation between aspirin resistance, determined by platelet aggregation and the composite primary outcome of death, miocardial infaction or cerebrovascular accident (18). In another study, of 105 patients with acute coronary syndrome who were included, 19% of the patients were aspirin resistant as determined by PFA-100. During the follow-up period, major cardiac adverse events occurred in 45% of the aspirin-resistant patients and in 11.7% of the patients with aspirin-sensitive platelets (19).

NON-COMPLIANCE AND ASPIRIN DOSES

Multiple causes have been proposed to explain the platelet resistance to aspirin. Among them, poor compliance is considered as one of the most important potential confounder factors in clinical outcome studies of aspirin resistance (20,21). For example, a study showed that 17 of 190 patients were aspirin resistant by light aggregometry, and 10 of 17 admitted that they had not been taking aspirin (22). Other studies have found around 29% of patients with aspirin resistence, and almost 50% of them admitted that they were not taking aspirin (23).

Another possible cause of aspirin resistance is the dose of aspirin used. In this regard, a work from Lee *et al.* (24) demonstrated that in patients with stable coronary artery disease who were taking 80 to 325 mg aspirin/day, the percent persistant was 30% in 384 patients taking 80–100 mg, 17% in 72 taking 150 mg and 0% in 12 patients taking 300 mg/day, suggesting that a 100 mg or less daily dose of aspirin, which may have lower side effects, is associated with a higher incidence of aspirin resistance. However, the comparison that higher doses of aspirin reduced aspirin resistance more than the lowest is not clear at all. Accordingly, the Antithrombotic Trialists Collaboration (25) demonstrated a tendency towards increased risk reduction in vascular events in the low dose (75–150 mg) groups as compared with a high dose (500–1,500 mg) group. In this line of knowledge, and although accumulative clinical and laboratory evidences suggest a reduced efficacy of aspirin in patients with diabetes mellitus, there were no clear additional clinical benefits in this patient population by increasing the aspirin dose. Moreover, a significant risk of gastrointestinal haemorrhage may occur with higher aspirin doses, and diabetic patients treated with higher aspirin dose may be exposed to a consideration risk that may outweigh the small benefits of such treatment. Only a limited number of studies have investigated the potential mechanisms involved in the higher aspirin resistance of platelets from diabetic patients. In diabetic patients, an increased level and activity of prothrombotic clotting factors was associated with a tight clot structure and an impaired fibrinolysis (26). These effects on clotting factors are largely due to insulin resistance, dyslipidaemia and low-grade inflammation (27). In this sense, interaction between glycation and acetylation has been shown (28,29), and increased glycation of platelet and coagulation factor

proteins may interfere with the acetylation process to contribute to aspirin resistance in diabetic patients (30).

Another possible reason for the variability in the platelet response to low-dose aspirin is the characterized drug-drug interaction with non-steroidal anti-inflammatory drugs (NSAIDs). Concomitant administration of aspirin with ibuprofen or naproxan is known to result in competition for a common docking site on Cyclooxygenase-1 (COX-1) that prevents aspirin from gaining access to, and acetylating, the target serine. This pharmacological interaction may be minimized if the patients take ibuprofen hours before aspirin or at least 30 min after taking aspirin.

GENETIC FACTORS OF ASPIRIN RESISTANCE

The gene encoding COX-1 protein is composed of 11 exons spanning a length of 22 Kb on chromosome 9. The promotor of COX-1 gene has multiple binding sites for transcription factors. The role of single nucleotide polymorphisms (SNPs) of COX-1 in the mechanism of aspirin resistance has been elucidated. In this regard, no difference was found in the frequency of SNPs in patients with recurrent stroke despite aspirin treatment when compared with controls (31). Another study demonstrated that patients who were heterozygous for A842G/C50T haplotype showed significantly greater inhibition of prostaglandin H2 formation by aspirin compared with common allele homozygotes (32). However, other studies found no association between COX-1 SNPs and the response to aspirin. Accordingly, a recent study developed by our group failed to find correlation between reduced aspirin response by platelets from patients with coronary artery disease and COX-1 SNPs in both the promotor (A-824G) and encoding regions (C22T and C50T) (33).

COX-1 NON-DEPENDENT MECHANISMS ASSOCIATED WITH ASPIRIN RESISTANCE

Alternative pathways to COX-1 inhibition have been also involved in the aspirin resistance syndrome. In this regard, upregulation of COX-2 in monocytes, macrophages and vascular endothelial cells has been described as a possible contributor to the aspirin resistance syndrome (34). COX-2 is present constitutively only in a limited number of cells and is inducible by activation of several signed transduction pathways. Platelets do not apparently express COX-2 except under special pathological conditions (35). COX-2 is covalently acetylated by aspirin at serine 516, but this does not stop the enzyme from oxidation of arachidonic acid. Acetylated COX-2 releases 15-hydroperoxide of eicosatetraenoic acid, a substrate for other eicosanoids mediators, i.e. 15-epi-lipoxins (35). The possibility that a variant COX-2 protein is present in platelets and plays a role in aspirin resistance has also been supported by studies of patients undergoing coronary artery bypass grafting in which postoperative aspirin resistance was paralleled by induction of COX-2 immunoreactivity in platelets, while platelet COX-1 protein expression was not changed after the surgery (36). Other studies have examined the possible role of COX-2 polymorphisms on response to aspirin. COX-2 −765C variant displayed a slightly higher reduction in 11-dTxB_2 level on treatment with aspirin (37). More recently, Censarek *et al.* cloned from platelet mRNA a novel COX-2 splice variant, designated COX-2a, which is characterized by a partial deletion of exon 5 that has been associated with aspirin resistance (38).

It is clear that the crosstalk between platelets and other cells included in the microvascular environment, i.e. leukocytes, the endothelium and even other platelets, is involved in the platelet response to aspirin. Therefore, it is plausible that other molecular mechanisms, to day unexplored, may contribute to the failure of aspirin to prevent platelet activation. For example, it has been postulated that elevated catecholamines and angiotensin II in patients with heart failure may be involved in the higher rate of aspirin resistance in this type of patients (39). Therefore, new technologies, as proteomics, may allow us to detect new molecular target and pathways involved in the platelet resistance to aspirin. Accordingly, we recently identified in the plasma from patients with stable coronary ischemia and with aspirin-resistant platelets a greater amount of three vitamin D binding protein (DBP) isotypes than plasma from stable coronary ischemic patients with aspirin-sensitive platelets (40). The main function of DBP is to bind and transport vitamin D analog. However, other emerging functions come to be attributed to DBP, including leukocyte activation. Additionally, *in vitro* studies suggested that DBP may modify the ability of aspirin to inhibit COX-1 activity in platelets since in the presence of DBP + aspirin, platelets produced greater amounts of TxB_2 than platelets incubated with aspirin alone (40). Therefore, DBP could be a circulating biomarker to identify coronary patients with platelets resistant to aspirin, although further studies are needed to assess it.

More and more evidence suggests that aspirin resistance may be related to platelet heterogeneity between individuals. In this regard, in patients receiving aspirin for secondary prevention of cardiovascular events, non-inhibited platelet COX activity persists more in younger and heavier patients (41). Moreover, it is known that smoking-enhanced platelet thrombosis is not prevented by aspirin treatment, while it has been associated with an increased platelet turnover (42). The elevated turnover of circulating platelets yields an

increased population of young platelets that are more reactive than the older platelet population. The young platelet population has been identified as reticulated platelets. Circulating reticulated platelets have been shown elevated in patients with acute coronary syndrome and stroke (41,43). Interestingly, Guthikonda *et al.* have recently demonstrated that reticulated platelets are associated with diminished antiplatelet effects of aspirin and increased aspirin resistance (44).

Using proteomics, we have also recently demonstrated the existence of a different level of expression of proteins in the platelets from aspirin-resistant patients than in those from aspirin-sensitive platelet from patients with stable coronary ischemia (45). Indeed, proteins associated with cytoskeleton, energetic metabolism, oxidative stress, inflammation and cell survival were analyzed. To discard non-compliance to aspirin in this work, it is noteworthy that all the included patients received an additional dose of aspirin (100 mg) 1 h before platelets were obtained. These results suggest that an increased expression of proteins associated with the apoptotic phenomenon occurs in platelets from aspirin-resistant patients, which may suggest an increased turnover of platelets in these patients. However, independently of the proteins and processes in which proteins may be involved, the main conclusion raised from this study was that platelets from aspirin-resistant and aspirin-sensitive patients are different in terms of the level of expression of some of the proteins that they expressed. A plausible hypothesis to explain the different levels of expression between them is that the type of platelets produced at megakaryocyte level could be different between aspirin-sensitive and aspirin-resistant patients. Therefore, the low and high responsiveness to aspirin by platelets could probably be designed during megakaryocytopoiesis and during platelet formation from mature megakaryocytes. Therefore, platelet resistance to aspirin could not only be related to aspirin but also to other antiplatelet drugs (Fig. 2). Therefore, the question then raised is if aspirin resistance could be treated and, if so, how.

TREATMENT OF ASPIRIN RESISTANCE

The most logical way to treat aspirin resistance is to identify and treat the underlying cause(s) of aspirin resistance.

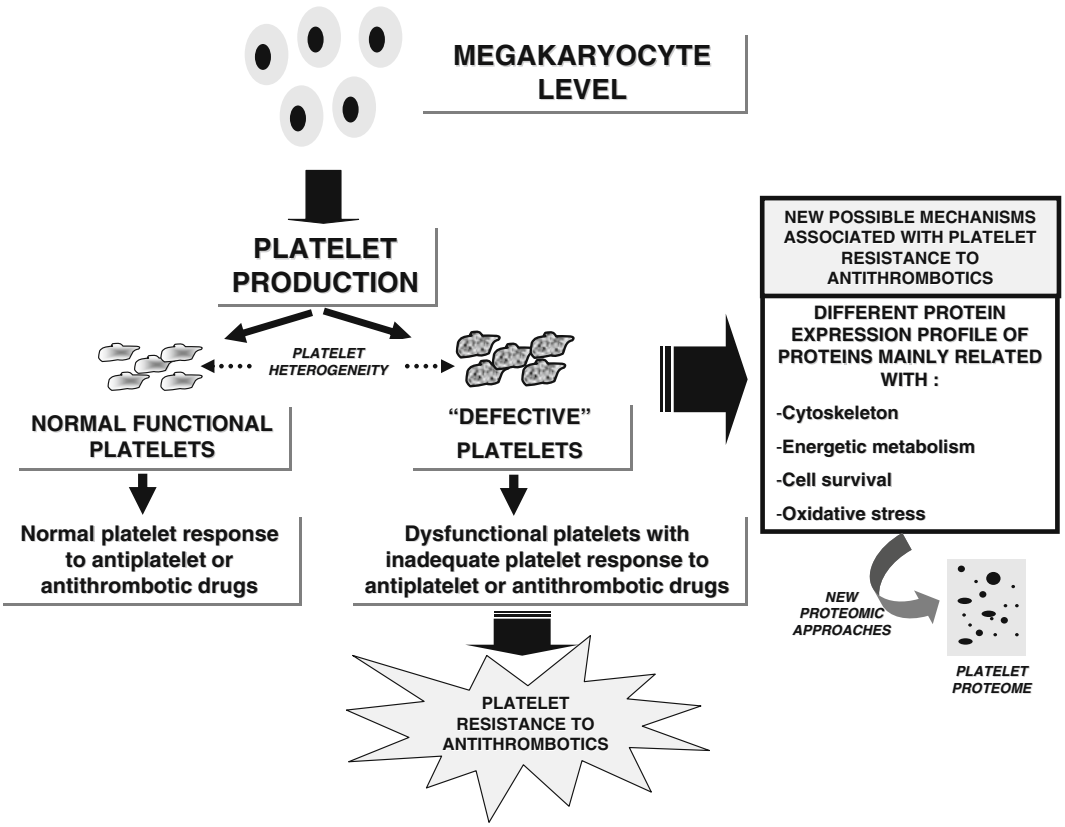

Fig. 2 Novel proposed mechanisms involved in platelet resistance to antithrombotic based on platelet heterogeneity. The type of platelets produced at the megakaryocyte level could be different among patients whose platelets are resistant or sensitive to antiplatelet drugs. These "dysfunctional" platelets have a different protein expression profile that could be involved in the low responsiveness to antiplatelet treatment shown by some cardiovascular patients. (Ref 45).

Potential effective treatments may include improvement of patient compliance with aspirin, avoidance of drugs that interact with aspirin, such as ibuprofen, smoking cessation and improved control of plasma glucose levels, the latter two of which both increase platelet turnover. Another possibility is to add other antithrombotic drugs with different downstream pathway than aspirin, e.g. clopidogrel. However, although all of them seem logical, they are not necessarily effective. In this regard, there is evidence that acute addition of clopidogrel to aspirin in patients during an acute coronary syndrome and undergoing percutaneous coronary interventions improves outcomes. However, long-term treatment with clopidogrel has not demonstrated additional benefits in aspirin-resistant patients (46,47), which may support that the molecular mechanisms associated with resistance of platelets to aspirin is not specific for aspirin but common to other antiplatelet drugs.

PLATELET RESISTANCE IS ONLY FOR ASPIRIN?

Antiplatelet therapy with aspirin and clopidogrel is the current treatment for patients with stable and unstable atherosclerotic cardiovascular disease, including those with diabetes mellitus. However, as above mentioned for aspirin, a portion of patients treated with dual therapy, aspirin and clopidogre, also experience recurrent atherothrombotic events. This opens the concept of clopidogrel resistance, which is determined *ex-vivo* by the ability of ADP to induce platelet activation in platelet from clopidogrel-treated patients. In this sense, clinical data have demonstrated a correlation between clopidogrel non-responsiveness and adverse clinical outcomes, including stent thrombosis (48,49). For example, 60 patients with ST elevation myocardial infarction undergoing primary PCI were stratified in quartiles based on the percent inhibition of ADP-induced platelet aggregation (50). Patients considered as resistant to clopidogrel (first quartile) showed a higher incidence of recurrent cardiovascular event.

Numerous molecular and cellular mechanisms have been postulated as being responsible for clopidogrel resistence. Upregulation of the P2Y12 pathway appears to be of particular importance in patients with type-2 diabetes mellitus (51). In this regard, a potential factor for clopidogrel resistance is the partial inhibition of platelet P2Y12 receptors. While there is no available data on the clopidogrel P2Y12 receptor occupancy rate, a binding study indicated that clopidogrel given as 75 mg/day for 10 days in healthy subjects reduced approximately by 60% the number of binding sites for ADP (52). The remaining binding sites that were insensitive to clopidogrel may either be located on the P2Y1 receptors or reflect clopidogrel´s incomplete P2Y12 receptors occupancy. However, to our knowledge, no studies have compared clopidogrel receptors' occupancy rate between non-responsive and responsive clopidogrel-treated patients.

Patient's body mass index (BMI) may be another contributing factor for the variability in the platelet response to clopidogrel. Overweight patients (body mass index$\geq$25 Kg/m^2) demonstrated a reduced antiplatelet effect with clopidogrel (53), which could be partially related to their propensity to insulin resistance.

Genetic mechanisms have been also involved in the reduced platelet response to clopidogrel. Polymorphisms of the P2Y12 receptor gene and polymorphisms of CYP3As have been included as causes of platelet resistance to clopidogrel (54). It is important to remember that clopidogrel is a pro-drug requiring oxidation by the hepatic cytochrome P450 (CYP450) to generate an active metabolite. CYP3A4 and CYP3A5 are the enzymes responsible for the oxidation of the thiophene ring of clopidogrel to finally form a disulfide bridge with the two extracellular cysteine residues located on the ADP P2Y12 receptor. This oxidation causes the irreversible blockade of ADP binding to platelets. In this regard, drugs that are metabolized by CYP3A4, like the acid form of atorvastatin, may interact with clopidogrel, inducing clopidogrel resistance (55). However, other investigators have refuted the relevancy of this interaction (56–58), and although the interaction between clopidogrel and statins like atorvastatin may exist, there is insufficient data to judge their clinical relevance.

TREATMENT OF PLATELET RESISTANCE TO CLOPIDOGREL

Several studies have focused on how to overcome clopidogrel non-responsiveness. Current guidelines provide a weak recommendation (class IIB, level of evidence C) for increasing the maintenance dose of clopidogrel to 150 mg/day. The use of a 150 mg clopidogrel maintenance dose resulted in marked platelet inhibition compared with 75 mg dose (59). However, most of these studies are not sufficiently powered to assess safety and clinical efficacy. The OPTIMUS-2 study showed that the increase in intraplatelet cyclic AMP levels induced by cilostazol led to enhance P2Y12 inhibitory effects (60). This may explain why triple oral antiplatelet therapy (aspirin, clopidogrel, cilostazol) is associated with better clinical outcomes than with dual therapy, particularly in patients with diabetes mellitus (61).

Taking into consideration the fact that platelet resistance to both aspirin and clopidogrel exists, a further possible antiplatelet treatment may be the use of novel antiplatelet agents such as prasugrel, a thyenopyridine of third generation that possess more potent and rapid effects than clopidogrel

and is not as dependent as clopidogrel on biotransformation to an active metabolite (62). In this regard, although the initial data seem to suggest that prasugrel increases the risk of bleeding, compared with clopidogrel, the PRINCIPLE-TIMI44 has shown that the dose of prasugrel used in TRITON leads to greater platelet inhibition than clopidogrel, at the higher loading and maintenance doses (63). Moreover, subgroup analysis of TRITON suggested that prasugrel may have greater benefit over clopidogrel in the highest-risk patients and in those with diabetes.

With respect to platelet thienopyridine resistance, a genomic substudy of the clopidogrel arm of the TRITON-TIMI 38 trial demonstrated that individuals treated with clopidogrel who carried the CYP2C19*2 genetic variant were at 1–5-fold higher risk for death, myocardial infarction, and stroke and at 3-fold higher risk for stent thrombosis compared with non-carriers (64). A lack of an association for these clinical endpoints in carriers of CYP2C19*2 who were treated with prasugrel supports that the cytochrome P450 genetic-related resistance to clopidogrel could not be similar for prasugrel. In this sense, it is remarkable that while CYP2C19 is important for clopidogrel activation, prasugrel is less dependent on CYP2C19 for its activation. However, our group has hypothesized that megakaryocytes may play a main role in the resistance to antiplatelet drugs. So, it is also possible that the low and high responsiveness to acute platelet treatment could be more specific for each individual patient. Therefore, new technologies, including genetic and proteomics, may allow for a better understanding of the mechanisms associated with resistance to antiplatelet therapy and even to individualize platelet inhibition in cardiovascular patients.

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REFERENCES

1. Antithrombotic Trialist´s Collaboration. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction and stroke in hihgh risk patients. BMJ. 2002;324:71–86.
2. Patrono C, Coller B, Dalen JE, FitzGerald GA, Fuster V, Gent M, *et al.* Platelet-active drugs: the relationship among dose, effectiveness and side effects. Chest. 2001;119:39S–63.
3. Physician's Health Study: aspirin and primary prevention of coronary heart disease. N Engl J Med. 1989;321:1825–8.
4. Antiplatelet Trialilst´s Collaboration. Collaborative overview of randomised trials of antiplatelet therapy–I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. BMJ. 1994;308:81–106.
5. ISIS-2 Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Lancet. 1988;2:349–60.
6. López-Farré A, Caramelo C, Esteban A, Alberola ML, Montón M, Millás I, *et al.* Effects of aspirin on platelets neutrophil interactions. Role of nitric oxide and endothelin-1. Circulation. 1995;91:2080–8.
7. Taubert D, Berkels R, Grosser N, Schröder H, Gründemann D, Schömig E. Aspirin induces nitric oxide release from vascular endothelium in a novel mechanism of action. Br J Pharmacol. 2004;143:159–65.
8. Steer KA, Wallace TM, Bolton CH, Hartog M. Aspirin protects low density lipoprotein from oxidative modification. Heart. 1997;77:333–7.
9. Husain S, Andrews NP, Mulcahy D, Panza JA, Quyyumi AA. Aspirin Improves Endothelial Dysfunction in Atherosclerosis. Circulation. 1998;97:716–20.
10. Santos MT, Valles J, Aznar J, Marcus AJ, Broekman MJ, Safer LB. Prothrombotic effects of erythrocytes on platelet reactivity. Reduction by aspirin. Circulation. 1997;95:63–8.
11. Michelson AD, Cattaneo M, Eikelboom JW, Grubel P, Kottke-Marchant K, Kunicki TJ, *et al.* Platelet physiology subcommittee of the scientific and standardization committee of the international society on thrombosis and haemostasis; working group on aspirin resistance. Aspirin resistance: position paper of the working group on aspirin resistance. J Thromb Haemost. 2005;3(6):1309–11.
12. Harrison P. Progress in the assessment of platelet function. Br J Haematol. 2000;111:73–734.
13. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after non.urgent percutaneous coronary intervention despite clopidogrel pretreatment. J Am Coll Cardiol. 2004;43:1122–6.
14. Hankey GJ, Eikelboom JW. Aspirin resistance. BMJ. 2004;328:477–9.
15. Bhatt DL. Aspirin resistance: more than just a laboratory curiosity. J Am Coll Cardiol. 2004;43:1122–6.
16. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post-stroke patients. Thromb Res. 1993;71:397–403.
17. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation. 2002;105:1650–5.
18. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. JACC. 2003;41:961–5.
19. Pamukcu B, Oflaz H, Oncul A, Umman B, Mercanoglu F, Ozcan M, *et al.* The role of aspirin resistance on outcome in patients with acute coronary syndrome and the effect of clopidogrel therapy in the prevention of major cardiovascular events. J Thromb Thrombolysis. 2006;22(2):103–10.
20. Cotter G, Shemesh E, Zehavi M, Dinur I, Rudnick A, Milo O, *et al.* Lack of aspirin effect: aspirin resistance or resistance to taking aspirin? Am Heart J. 2004;147:293–300.
21. Carney RM, Freeland KE, Eisen SA, Rich MW, Skala JA, Jaffe AS. Adherence to a prophylactic medication regimen in patients with symptomatic *versus* asymptomatic ischemic heart disease. Behav Med. 1998;24:35–9.
22. Schwartz KA, Schwartz DE, Ghosheh K, Reeves MJ, Barber K, DeFranco A. Compliance as a critical consideration in patients who appear to be resistant to aspirin after healing of myocardial infarction. Am J Cardiol. 2005;95:973–5.

23. Cotter G, Shemesh E, Zehavi M, Dinur I, Rudnick A, Milo O, *et al.* Lack of Aspirin effect: aspirin resistance or resistance to taking aspirin? Am Heart J. 2004;147:293–300.
24. Lee PY, Chen WH, Ng W, Cheng X, Kwok JY, Tse HF, *et al.* Low-dose, aspirin increases aspirin resistance in patients with coronary artery disease. Am J Med. 2005;118:723–7.
25. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, *et al.* Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373:1849.
26. Dunn EJ, Phillipou H, Ariens R, Grant PJ. Molecular mechanisms involved in the resistance of fibrin to clot lysis by plasmin in subjects with type 2 diabetes mellitus. Diabetologia. 2006;49:1071–80.
27. Ajjan R, Grant PJ. Coagulation and atherombotic disease. Atherosclerosis. 2006;186:240–59.
28. Rendell M, Nierenberg J, Brannan C, Valentine JL, Stephen PM, Dodds S, *et al.* Inhibition of glycation of albumin and hemoglobin by acetylation *in vitro* and *in vivo*. J Lab Clin Med. 1986;108:286–93.
29. Swamy-Mruthini S, Carter AL. Acetyl-L-carnitine decreases glycation of lens proteins: *in vitro* studies. Exp Eye Res. 1999;69:109–15.
30. Watala C, Pluta J, Golanski J, Rozalski M, Czyz M, Trojanowski Z, *et al.* Increased protein glycation in diabetes mellitus is associated with decreased aspirin-mediated protein acetylation and reduced sensitivity of blood platelets to aspirin. J Mol Med. 2005;83:148–58.
31. Hillarp A, Palmquist B, Lethagen S, Villoutreix BO, Mattiasson I. Mutations within the cyclooxygenase-1 gene in aspirin non-responders with recurrence of stroke. Thromb Res. 2003;112:275–83.
32. Halushka MK, Walker LP, Halushka PV. Genetic variation in cyclooxigenase 1: effects on response to aspirin. Clin Pharmacol Ther. 2003;73:122–30.
33. Mateos-Cáceres PJ, Azcona L, Fernández-Ortiz A, Sacristán D, Bernardo E, Ramos-Mozo P, Alonso-Orgaz S, Fernández-Arquero M, López-Farré A, Macaya C. Proteomics and genetics aspects of aspirin resistance. Trauma Fund MAPFRE. 2008.
34. Schonbeck U, Sukhova GK, Graber P, Coulter S, Libby P. Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions. Am J Pathol. 1999;155:1281–9.
35. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998;38:97–120.
36. Censarek P, Freídle K, Udelhoven M, Ku SJ, Hohlfeld T, Meyer-Kirchrath JJ, *et al.* Cyclooxygenase COX-2a, a novel COX-2 mRNA variant, in platelets from patients after coronary artery bypass grafting. Thromb Haemost. 2004;92:925–9288.
37. González-Conejero R, Rivera J, Corral J, Acuña C, Guerrero JA, Vicente V. Biological assessment of aspirin efficacy on healthy individuals: heterogeneus response to aspirin failure? Stroke. 2005;36:276–80.
38. Censarek P, Steger G, Paolini C, Hohlfeld T, Grosser T, Zimmermann N, *et al*. Alternative splicing of platelet cyclooxigenase-2 mRNA in patients after coronary artery bypass grafting. Thromb Haemost. 2007;98:1309–15.
39. Sane DC, McKee SA, Malinin AI, Serebruany VL. Frecuency of aspirin resistance in patients with congestive heart failure treated with antecedent aspirin. Am J Cardiol. 2002;90:893–5.
40. López-Farré AJ, Mateos-Cáceres PJ, Sacristán D, Azcona L, Bernardo E, Pérez de Prada T, *et al.* Relationship between vitamina binding protein and aspirin resistance in coronary ischemic patients: A proteomic study. J Proteome Res. 2007;6:2481–7.
41. Lakkis N, Dokainish H, Abuzahra M, Tsyboulev V, Jorgensen J, Ponce De Leon A, *et al.* Reticulated platelets in acute coronary syndrome: A marker of platelet activity. J Am Coll Cardiol. 2004;44:2091–3.
42. Hung J, Lam JYT, Lacoste L, Letchacovski G. Cigarette smoking acutely increases platelet thrombus formation in patients with coronary artery disease taking aspirin. Circulation. 1995;92:2432–6.
43. Nakamura T, Uchiyama S, Yamazaki M, Okubo K, Takakuwa Y, Iwata M. Flow cytometric analysis of reticulated platelets in patients with ischemic stroke. Thromb Res. 2002;106:171–7.
44. Guthikonda S, Lev EI, Patel R, Delao T, Bergeron AL, Dong J-F, *et al.* Reticulated platelets and uninhibited COX-1 and COX-2 decrease the antiplatelet effects of aspirin. Thromb Haemost. 2007;5:490–6.
45. Mateos-Cáceres PJ, Macaya C, Azcona L, Modrego J, Mahillo E, Bernardo E, *et al.* Different expression of proteins in platelets from aspirin-resistant and aspirin-sensitive patients. Thromb Haemost. 2010;103(1):160–70.
46. Chen WH, Lee PY, William NG, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. J Am Coll Cardiol. 2004;43:1122–6.
47. Prabhakaran S, Wells KR, Lee VH, Flaherty CA, Lopes DK. Prevalence and Risk Factors for Aspirin and Clopidogrel Resistance in Cerebrovascular Stenting. Am J Neuroradiol. 2008;29:281–5.
48. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, *et al.* Variability in individual responsiveness to clopidogrel: clinical implications, management, and future presperctives. JACC. 2007;49:1505–16.
49. Angiolillo DJ, Guzman LA, Bass TA. Current antiplatelet therapies: benefits and limitations. Am Heart J. 2008;156(2 Suppl):S3–9.
50. Matetzky S, Shenkman B, Guetta V, Shechter M, Bienart R, Goldenberg I, *et al.* Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation. 2004;109:3171–5.
51. Ferreira IA, Mocking AIM, Feijge MAH, Gorter G, van Haeften TW, Heemskerk JWM, *et al.* Platelet Inhibition by Insulin Is Absent in Type 2 Diabetes Mellitus. Arterioscler Thromb Vasc Biol. 2006;26:417–22.
52. Mills DC, Puri R, Hu CJ, Minniti C, Grana G, Freedman MD, *et al.* Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. Arterioscler Thromb. 1992;12:430–6.
53. Angiolillo DJ, Fernández-Ortiz A, Bernardo E, Barrera Ramírez C, Sabaté M, Fernandez C, *et al.* Platelet aggregation according to body mass index in patients undergoing coronary stenting: should clopidogrel loading-dose be weigh adjusted? J Invasive Cardiol. 2004;16:169–74.
54. Wiviott SD, Antman EM. Clopidogrel resistance: a new chapter in a fast-moving story. Circulation. 2004;109:3064–7.
55. Jacobsen W, Kuhn B, Soldner A, Kirchner G, Sewing KF, Kollman PA, *et al.* Lactonization is the critical first step in the disposition of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor atorvastatin. Drug Metab Dispos. 2000;28:1369–78.
56. Serebruany VL, Malinin AI, Callahan KP, Gurbel PA, Steinhubl SR. Statins do not affect platelet inhibition with clopidogrel during coronary stenting. Atherosclerosis. 2001;159:239–41.
57. Wienbergen H, Gitt AK, Schiele R, Juenger C, Heer T, Meisenzahl C, *et al.* Comparison of clinical benefits of clopidogrel therapy in patients with acute coronary syndromes taking atorvastatin *versus* other statin therapies. Am J Cardiol. 2003;92:285–8.
58. Gorchakova O, von Beckerath N, Gawaz M, Mocz A, Joost A, Schömig A, *et al.* Antiplatelet effects of a 600 mg loading dose of clopidogrel are not attenuated in patients receiving atorvastatin or simvastatin for at least4 weeks prior to coronary artery stenting. Eur Heart J. 2004;25:1898–902.
59. Angiolillo DJ, Shoemaker SB, Desai B, Yuan H, Charlton RK, Bernardo E, *et al.* A randomized comparison of a high clopidogrel

maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the OPTIMUS (Optimizing anti-Platelet Therapy In diabetes MellitUS) study. Circulation. 2007;115:708–16.
60. Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, *et al.* A randomize study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. Eur Heart J. 2008;29:2202–11.
61. Lee SW, Park SW, Kim YH, Yun SC, Park DW, Lee CW, Hong MK, Kim HS, Ko JK, Park JH, Lee JH, Choi SW, Seong IW, Cho YH, Lee NH, Kim JH, Chun KJ, Park SJ. Drug-eluting stenting followed by cilostazol treatment reduces late restenoses in patients with diabetes mellitus the DECLARE DIABETES Trial (A Randomized Comparison of Triple Antiplatelet Therapy with Dual Antiplatelet Therapy After Drug.Eluting Stent Implantation in Diabetic Patients). J Am Coll Cardiol. 2008;1181–7.
62. Wiviott SD, Antman EM, Winters KJ, Weerakkody G, Murphy SA, Behounek BD, *et al.* JUMBO-TIMI 26 Investigators. Randomized comparison of prasugrel (CS-747, LY640315), a novel thienopyridine P2Y12 antagonist, with clopidogrel in percutaneous coronary intervention: results of the Joint Utilization of Medications to Block Platelets Optimally (JUMBO)-TIMI 26 trial. Circulation. 2005;111:3366–73.
63. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, *et al.* Prasugrel compared with high loading and maintenance-dose clopidogrel in patients with planned percutaneus coronary intervention; the Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation. Thrombolysis in Myocardical Infarction 44 trial. Circulation. 2007;116:2923–32.
64. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, *et al.* Cytochrome P-450 plymorphisms and response to clopidogrel. N Engl J Med. 2009;360:354–62.