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STATE-OF-THE-ART PAPER

Resistance to Clopidogrel: A Review of the Evidence

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Current available data show that about 4% to 30% of patients treated with conventional doses of clopidogrel do not display adequate antiplatelet response. Clopidogrel resistance is a widely used term that remains to be clearly defined. So far, it has been used to reflect failure of clopidogrel to achieve its antiaggregatory effect. The interpatient variability in clopidogrel response is multifactorial. It can be due to extrinsic or intrinsic mechanisms. Among extrinsic mechanisms are the possibility of clopidogrel underdosing in patients undergoing stenting or with acute coronary syndrome, and drug-drug interactions involving CYP3A4. Intrinsic mechanisms include genetic polymorphisms of the $P2Y_{12}$ receptor and of the CYP3As, accrued release of adenosine diphosphate, or up-regulation of other platelet activation pathways. Presently, there is no definite demonstration of an association between low responsiveness to clopidogrel and thrombotic events. The optimal level of clopidogrel-induced platelet inhibition, which will correlate quantitatively with clopidogrel's ability to prevent atherothrombotic events is still lacking. Furthermore, because there is no single and validated platelet function assay to measure clopidogrel's antiplatelet effect, it is not justified to routinely look for clopidogrel resistance in the clinical setting. This review discusses currently available evidence surrounding the variability in the antiplatelet response to clopidogrel. (J Am Coll Cardiol 2005;45:1157–64) © 2005 by the American College of Cardiology Foundation

Platelet activation and aggregation play an important role in the pathogenesis of arterial thrombosis leading to acute coronary syndromes (ACS) and in thrombotic complications during and after percutaneous coronary interventions (PCI) (1,2). When used alone, clopidogrel has been proven effective in preventing ischemic events in patients with atherosclerotic vascular disease (3). In combination with aspirin, clopidogrel is the gold standard for the prevention of subacute stent thrombosis (SAT) in patients undergoing PCI and reduces major adverse cardiovascular events in patients with non–ST-segment elevation ACS (4,5). Aspirin-resistant patients may also benefit from clopidogrel due to their increased platelet sensitivity to adenosine diphosphate (ADP) (6). However, clopidogrel's antiplatelet effect is not uniform in all patients (7–11).

The concept of clopidogrel resistance is increasingly evoked in the literature in light of the newly acknowledged variability in the antiplatelet response. However, there is currently no clear and consensual definition of what clopidogrel resistance means. This article discusses the magnitude and the potential causes of low responsiveness to clopidogrel by presenting a comprehensive review of currently available evidence.

PLATELET AGGREGATION

Adenosine diphosphate plays an important role in platelet activation and aggregation (12,13). Upon damaged or disrupted endothelium, circulating platelets adhere to the vessel wall through interactions with the subendothelium constituents (collagen, von Willebrand factor, and other adhesive proteins such as fibronectin, laminin, and vitronectin) (13,14). After adhesion, these anchored platelets undergo conformational changes through the action of several extrinsic activators such as collagen, thrombin, and epinephrine. Once activated, platelets release the contents of their dense (platelet agonists such as ADP and serotonin) and alpha-granules (fibrinogen, von Willebrand factor, other adhesive proteins, proinflammatory factors, and prothrombotic factors), which trigger platelet-activating intracellular signals in surrounding platelets. Activated platelets also synthesize and release thromboxane A_2 in circulation. Activated and degranulated platelets expose glycoprotein (GP) IIb/IIIa receptors at their surface allowing fibrinogen binding, which forms bridges between adjacent activated platelets causing platelet aggregation. In addition, the release of granule contents amplifies the coagulation and inflammatory processes.

ADP and its receptor. Adenosine diphosphate binds to neighboring platelets through two G-protein coupled receptors ($P2Y_1$ and $P2Y_{12}$) and the cation channel-coupled $P2X_1$ receptor (Fig. 1) (15). The activation of $P2X_1$ receptor mediates a rapid transient calcium ion influx in platelets but does not play a major role in platelet aggregation (15). Stimulation of the G_q-coupled $P2Y_1$ receptor activates phospholipase C and induces a transient rise in cytosolic calcium resulting in a platelet conformational change and in weak, transient platelet aggregation (16). Activation of the G_i-coupled $P2Y_{12}$ receptor by ADP liberates the G_i protein subunits α_{Gi} and $\beta\gamma$, which couple to independent signaling

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Abbreviations and Acronyms

ACS	= acute coronary syndromes
ADP	= adenosine diphosphate
cAMP	= cyclic adenosine monophosphate
CYP450	= cytochrome P450
GPIIb/IIIa	= glycoprotein IIb/IIIa
PCI	= percutaneous coronary intervention
PGE_1	= prostaglandin E_1
SAT	= subacute stent thrombosis
VASP	= vasodilator-stimulated phosphoprotein

events and lead to a sustained platelet aggregation (17). The subunit α_{Gi} decreases the platelet cyclic adenosine monophosphate (cAMP) level through the inhibition of adenylyl cyclase. This decrease in cAMP production leads, in turn, to a reduction in the activation of specific protein kinases, which can no longer phosphorylate the vasodilator-stimulated phosphoprotein (VASP); VASP phosphorylation is crucial for GP IIb/IIIa receptor inhibition (17). The subunit $\beta\gamma$ activates the phosphatidylinositol 3-kinase, which is an important signaling molecule for $P2Y_{12}$-mediated platelet-dense granule secretion and GP IIb/IIIa receptor activation (18). Signaling events downstream of the $P2Y_{12}$ receptor mediate thromboxane A_2 production, α-granule release, and subsequent expression of P-selectin on activated platelets (19). The $P2Y_{12}$ receptor is also involved in thrombus growth and stability (18). Stimulation of both $P2Y_1$ and $P2Y_{12}$ receptors is required to cause ADP-induced platelet aggregation (15). Interestingly, epinephrine, which represses cAMP levels through its $alpha_{2A}$-adrenergic receptor, can produce certain, but not all, features of $P2Y_{12}$ signaling (18).

MECHANISM OF ACTION AND PHARMACOKINETICS OF CLOPIDOGREL

Clopidogrel, an ADP-receptor antagonist, is a prodrug requiring oxidation by the hepatic cytochrome P450

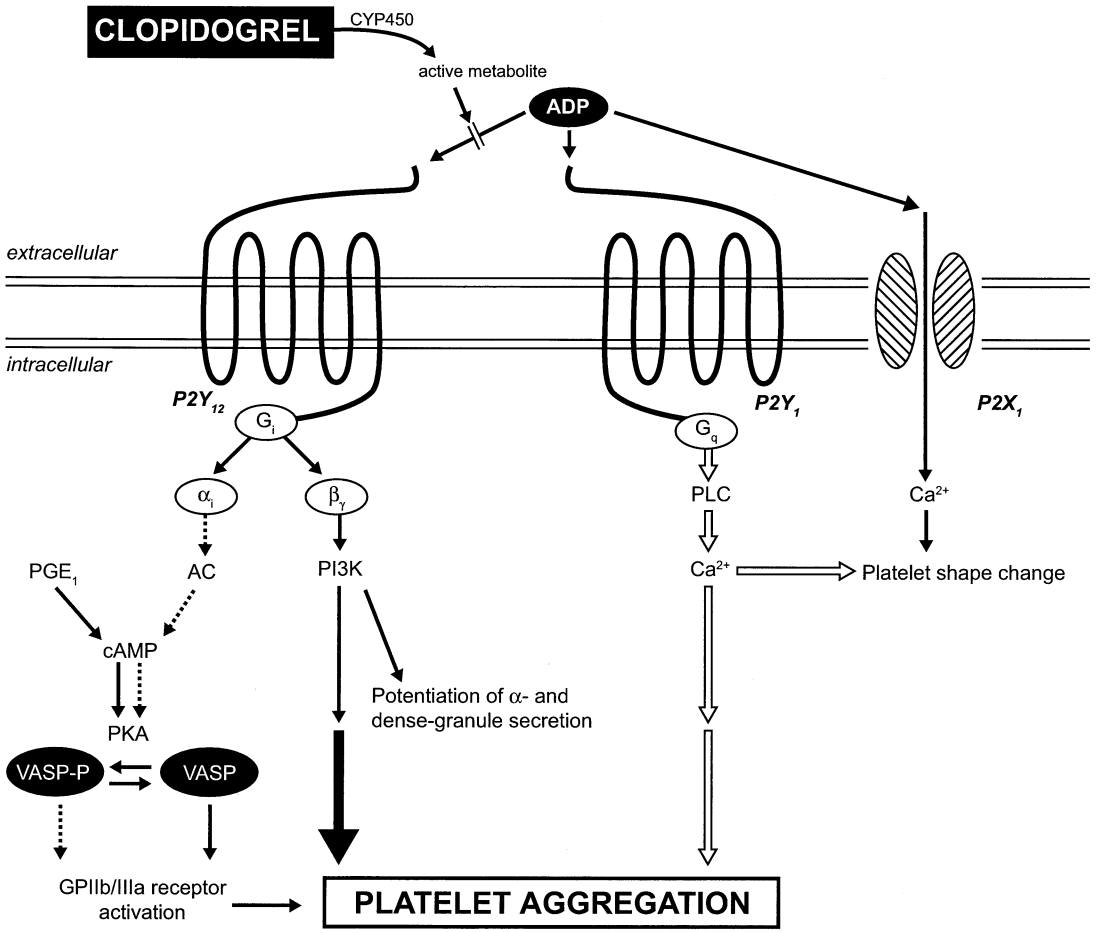

Figure 1. Mechanism of action of clopidogrel. Clopidogrel competitively and irreversibly inhibits the adenosine diphosphate (ADP) $P2Y_{12}$ receptor; ADP binds to the $P2Y_1$ receptor to induce change in platelet shape and a weak and transient platelet aggregation. The binding of ADP to its G_i-coupled $P2Y_{12}$ receptor liberates the G_i protein subunits α_{Gi} and β_γ. The subunit α_{Gi} leads to the inhibition of adenylyl cyclase (AC), which, in turn, lowers the cyclic adenosine monophosphate (cAMP) level. This inhibits the cAMP-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP) (VASP-P), which is known to be closely related to the inhibition of glyprotein IIb/IIIa receptor activation. The subunit β_γ activates the phosphatidylinositol 3-kinase (PI3K), which potentiates dense- and α-granule secretion. Multiple **arrows** within a given pathway indicate that intermediate steps may be involved. **Dotted arrows** indicate inhibition, whereas **solid arrows** represent activation. CYP450 = cytochrome P450; PGE_1 = prostaglandin E_1; PKA = protein kinase activation; PLC = phospholipase C.

(CYP450) to generate an active metabolite (20,21). Only a small proportion of clopidogrel undergoes metabolism by CYP450; it is mostly hydrolyzed by esterases to an inactive carboxylic acid derivative that accounts for 85% of clopidogrel-related circulating compounds. CYP3A4 and CYP3A5 are the enzymes responsible for the oxidation of the thiophene ring of clopidogrel to 2-oxoclopidogrel, which is further oxidized, resulting in the opening of the thiophene ring and the formation of both a carboxyl and a thiol group (20,21). The latter forms a disulfide bridge with the two extracellular cysteine residues located on the ADP $P2Y_{12}$ receptor expressed on the platelet surface and causes an irreversible blockade of ADP binding for the platelet's life span (22).

A standard dose of clopidogrel will achieve incomplete $P2Y_{12}$ antagonism, which translates into approximately 50% inhibition of ADP-induced platelet aggregation (23). The clopidogrel-induced platelet inhibition is dose- and time-dependent. In healthy subjects, platelet inhibition is dose-related up to a single dose of 400 mg, with no further increase with 600 mg (24). The maximum inhibition obtained with a single 400-mg dose is achieved after 2 to 5 h, whereas a daily dose of 75 mg takes 3 to 7 days to reach the same level of inhibition (25).

In addition to its antiaggregatory effect, clopidogrel attenuates the increase in C-reactive protein and decreases the expression of activated platelet-dependent inflammatory markers, namely CD40 ligand and CD62 P-selectin, which occur in patients undergoing PCI (26,27). The CD40 ligand is a potent stimulus of vascular inflammation, which promotes platelet-leukocyte interactions and induces tissue factor expression. Clopidogrel also reduces the formation of platelet-leukocyte conjugates in patients with non–ST-segment elevation ACS (28).

PREVALENCE AND CONTRIBUTING FACTORS TO CLOPIDOGREL RESISTANCE

Clopidogrel-induced platelet inhibition is patient-specific (7,8). The concept of clopidogrel resistance has emerged in the medical literature to reflect the failure to inhibit platelet function in vitro, although its existence and definition remain to be established. It has been proposed that the term *resistance* encompasses patients for whom clopidogrel does not achieve its pharmacological effect, and *failure of therapy* reflects patients who have recurrent events on therapy (29).

The prevalence of clopidogrel nonresponse in patients is evaluated between 4% and 30% 24 h after administration (7,8,10,30–32). The reported rates vary between studies because of the technique used to measure the extent of platelet aggregation and the presence of factors contributing to greater baseline platelet reactivity. Furthermore, the definition of nonresponders is not standardized.

Platelet function assays. Platelet function is measured in vitro, in most instances, by light transmission aggregometry. Although this method is still considered as the gold standard, it carries some disadvantages such as limited reproducibility and complex sample preparation (33,34). Other techniques are available, but not all are adequate to monitor clopidogrel antiaggregatory effect (34).

Adenosine-diphosphate-induced platelet aggregation is widely used to measure the effect of clopidogrel, but it may not be the most suitable test. Although clopidogrel prevents ADP from promoting platelet aggregation via its $P2Y_{12}$ receptor, ADP can still induce a transient platelet aggregation via its $P2Y_1$ receptor (15). Furthermore, the extent of $P2Y_1$-dependent platelet aggregation may vary widely among patients taking clopidogrel. A more specific way to assess the antiaggregatory effect of clopidogrel would be to measure the extent of ADP-induced inhibition of adenylyl cyclase, which is mediated uniquely by the $P2Y_{12}$ receptor (35).

Also, ADP concentrations used to induce platelet aggregation influence the detected rate of nonresponders, which will be increased with higher ADP concentrations (10). Furthermore, use of a low concentration of ADP (e.g., 1 μM) can only elicit the primary, and rapidly reversible, phase of platelet aggregation, a phase that is easily inhibited but may not be clinically meaningful (7,30,36).

The time chosen to measure platelet aggregation is also of paramount importance because a 300-mg loading dose of clopidogrel can only elicit its full antiplatelet effect at 24 h in contrast with a 600-mg loading dose, which can achieve its maximum effect after only 4 h (8,37,38). In one study, roughly half of the patients who were considered nonresponders at 24 h postloading of clopidogrel became responders by 30 days (9). This finding may be attributed to the weaning of early post-stenting platelet activation as time accrues after the procedure.

Presently, the validity of the reported rates of clopidogrel nonresponders is undermined by the absence of a consensual and justified platelet aggregation inhibition cutoff value to identify nonresponders to clopidogrel. Authors have used empirically defined cutoff values varying between <10% to <40% to segregate nonresponders from responders (7,8,10,30,31).

These arbitrary cutoff values do not make sense clinically because the optimal level of inhibition of platelet aggregation to prevent cardiovascular events may vary upon the clinical situation. While there is no available data on the clopidogrel $P2Y_{12}$ 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 (39). The remaining binding sites that were insensitive to clopidogrel may either be located on the $P2Y_1$ receptors or reflect clopidogrel's incomplete $P2Y_{12}$ receptor occupancy. So far, no study has compared clopidogrel receptor occupancy rate between nonresponsive and responsive patients. In a porcine model of stent thrombosis, clopidogrel produced a dose-dependent inhibition of stent thrombosis (40). A dose of clopidogrel producing 40% inhibition of ADP-induced platelet aggregation reduced by

56% the stent clot weight, whereas a higher dose reaching 80% platelet inhibition decreased the clot weight by 86%. One can speculate that, in a high-risk clinical situation, the required level of platelet inhibition should be higher than in a low-risk clinical situation.

Contributing factors to greater baseline platelet reactivity. High pretreatment platelet reactivity and thrombotic burden may contribute to a lower clopidogrel antiplatelet effect as these patients remain the most reactive during the first five days of clopidogrel treatment (8). Similarly, a 450-mg clopidogrel loading dose given ≥3 h before PCI resulted in a lower inhibition of platelet aggregation in patients with a higher unstable angina class on presentation as compared with patients having stable angina or lower unstable angina class (19 ± 22% vs. 32 ± 22%; p = 0.0044) (11). Patient's body mass index may be another contributing factor to the variability in platelet response to clopidogrel. Overweight patients (body mass index ≥25 kg/m^2), due in part to their propensity to insulin resistance, demonstrated a reduced antiplatelet effect with clopidogrel (31,41). However, this data needs to be confirmed because of the small sample size and nonrandomized design of the study.

A recent study in the context of elective PCI suggested that postclopidogrel platelet reactivity is more related to baseline platelet reactivity than to clopidogrel responsiveness (42). About 16% of patients with moderate-to-high baseline platelet reactivity remained with moderate post-treatment reactivity despite being responsive to clopidogrel.

Considering all the factors that may affect platelet aggregation, it is of paramount importance to measure baseline value and report percent change from baseline rather than using an arbitrary cutoff.

CLINICAL RELEVANCE OF CLOPIDOGREL RESISTANCE

Only two small trials explored the clinical relevance of an inadequate platelet response to clopidogrel (43,44). Their authors suggested that clopidogrel nonresponders may be at higher risk for thrombotic events.

Barragan et al. (43) demonstrated that patients experiencing SAT had a significantly higher platelet reactivity at the time of intervention for stent thrombosis compared with their counterparts who did not have a SAT but were receiving similar antiplatelet pretreatment. This study, however, did not separately analyze patients taking clopidogrel from those taking ticlopidine. Finally, platelet aggregation level was not assessed at baseline for either group. Given the high interindividual variability in platelet reactivity and in laboratory test responses, it is mandatory to compare the results obtained before and after clopidogrel administration. Otherwise, this may lead to inaccurate and paradoxical interpretation of the findings (35).

Interestingly, Barragan et al. (43) used a flow cytometric assay based on the quantification of the VASP phosphorylation state to evaluate thienopyridine's antiplatelet effect. This assay exploited the rationale that the level of VASP phosphorylation is closely related to the degree of inhibition of ADP-induced GP IIb/IIIa receptor activation and is, therefore, more specific to clopidogrel (Fig. 1). The phosphorylation of VASP is stimulated by the prostaglandin E_1 (PGE_1) via the increase in platelet cAMP level. In contrast, ADP inhibits PGE_1-stimulated VASP phosphorylation by lowering the cAMP level through its effects on the $P2Y_{12}$ receptor. The VASP phosphorylation flow cytometry assay is performed by incubating blood samples in vitro with ADP and/or PGE_1 before immunolabeling them with a monoclonal antibody directed against serine 239-phosphorylated VASP. Mean fluorescence intensity corresponding to each experimental condition, resting (PGE_1 alone) and activated platelets (ADP + PGE_1) is determined to establish a ratio that directly correlates with the VASP phosphorylation state and is expressed as a mean percentage of platelet reactivity. Clopidogrel does not alter the basal and PGE_1-stimulated VASP phosphorylation but strongly attenuates the inhibitory effect of ADP on PGE_1-stimulated VASP phosphorylation (17). A fall in VASP phosphorylation after stimulation by ADP indicates incomplete inhibition of the $P2Y_{12}$ receptor by clopidogrel.

In the second study, 60 patients with ST-segment elevation myocardial infarction undergoing primary PCI were stratified in quartiles based on their percent inhibition of ADP-induced platelet aggregation at day 6 compared to baseline (44). Patients in the first quartile (n = 15) were considered resistant to clopidogrel (ADP-induced platelet aggregation = 103 ± 8%). Patients in the second quartile through fourth quartile showed an increasing response to clopidogrel (69 ± 3%, 58 ± 7%, and 33 ± 12%, respectively). Six of the 15 patients (40%) in the first quartile developed a recurrent cardiovascular event (ST-segment elevation myocardial infarction, SAT, ACS, and acute peripheral arterial occlusion) through the six-month follow-up period, whereas only 1 patient of 15 (6.7%) in the second quartile experienced an event. No recurrent cardiac event occurred in patients in the third and fourth quartiles. However, the small sample size and the low number of recurrent cardiovascular events made this study underpowered and precluded any strong conclusions.

In brief, before any definitive conclusion can be drawn about the clinical consequences of low antiplatelet response to clopidogrel, these results need to be reproduced in a larger set of patients. Inhibition of platelet aggregation remains to be correlated efficiently with clinical outcomes. For this to occur, the platelet function assay has to be specific to clopidogrel effect and give reproducible results. Of note, the standard dosing regimen of clopidogrel used for coronary stenting was derived mainly from studies involving healthy volunteers and stable patients with coronary artery disease not undergoing stenting (45). The dose of clopidogrel selected in clinical trials was equivalent to ticlopidine 250 mg twice daily in terms of platelet inhibition and bleeding time. However, in the context of PCI or ACS where the thrombotic burden is heightened, the standard

Table 1. Potential Mechanisms of Clopidogrel Resistance

Extrinsic mechanisms
1. Patient non-compliance
2. Under-dosing or inappropriate dosing of clopidogrel
3. Drug-drug interactions involving CYP3A4
Intrinsic mechanisms
1. Genetic variables
a. Polymorphisms of $P2Y_{12}$ receptor
b. Polymorphisms of CYP3As
2. Increase release of ADP
3. Alternate pathways of platelet activation:
a. Failure to inhibit catecholamine-mediated platelet activation (epinephrine)
b. Greater extent of $P2Y_1$-dependent platelet aggregation
c. Up-regulation of $P2Y_{12}$-independent pathways (thrombin, thromboxane A_2, collagen)

ADP = adenosine diphosphate; CYP3As = cytochrome P450 3As.

dose of clopidogrel may be insufficient and lead to a breakthrough effect.

Importantly, the relative contribution of the anti-inflammatory properties of clopidogrel to its clinical benefit has not been weighted against its antiplatelet effect. In patients nonresponsive to clopidogrel, the importance of this anti-inflammatory effect has not been assessed.

If faced with a time constraint, one approach to increase the response to clopidogrel may be to give a higher loading dose of clopidogrel such as 600 mg, which will provide a faster and greater inhibition of platelet aggregation than the standard 300-mg loading dose (10,46). Another option would be to use a higher transient maintenance dose during critical, high-risk period (e.g., after PCI). However, despite the improvement in clopidogrel platelet inhibitory effect, the high loading dose does not eliminate interindividual variability in clopidogrel response (46). This calls for a contributing role of other factors beside drug dose to this phenomenon. Whether a high clopidogrel loading dose would be of any value in patients nonresponsive to a standard dosing regimen remains to be established.

POTENTIAL MECHANISMS OF CLOPIDOGREL RESISTANCE

Several mechanisms of clopidogrel resistance are possible and can be classified into two categories: extrinsic and intrinsic (Table 1).

Extrinsic mechanisms that reflect a reduced bioavailability of clopidogrel may include noncompliance to clopidogrel therapy, underdosing, or inappropriate dosing of clopidogrel, and drug-drug interactions affecting the biotransformation of clopidogrel into its active metabolite (29).

Possible intrinsic mechanisms may include polymorphisms of the $P2Y_{12}$ receptor gene leading to an increased number of $P2Y_{12}$ receptors, and polymorphisms of the CYP3As (29). There are also possibilities of increased release of ADP and up-regulation of other platelet activation pathways (such as thrombin, ADP, collagen, thromboxane A_2, and epinephrine) (29).

Drug-drug interactions. Any medication inhibiting or being a substrate of the CYP3A4 can potentially block the conversion of clopidogrel into its active metabolite. Among these medications figure hydroxymethylglutaryl-CoA reductase inhibitors, commonly referred to as statins. Most commercially available statins are lipophilic, with the exception of pravastatin, and must be metabolized, usually hepatically, to hydrophilic derivatives before they can be excreted through the kidney. Lovastatin and simvastatin show moderate affinity, whereas atorvastatin demonstrates a lower affinity to CYP3A4 (47,48). Fluvastatin and rosuvastatin are substrates of CYP2C9 (47).

Currently available atorvastatin is an active acid form and a weak substrate for CYP3A4. However, this acid form is rapidly converted to its lactone form by numerous enzymatic processes and has a significantly higher binding affinity for CYP3A4 than the acid form (49). The interaction between atorvastatin and clopidogrel was first revealed using genetically engineered microsomes of human CYP3A4 and CYP3A5 showing atorvastatin lactone greatly inhibited (90%) CYP3A4-dependent clopidogrel metabolism, more so than the atorvastatin acid form (20).

Lau et al. (50) reiterated the relevancy of this interaction by demonstrating that atorvastatin, at a dose of 40 mg, produced a dose-dependent and statistically significant reduction in the antiplatelet effect of clopidogrel, which remained significant six to eight days after stent implantation. In contrast, pravastatin, a non–CYP3A4-metabolized statin, did not influence the level of clopidogrel-induced platelet inhibition in patients undergoing successful PCI. Although interesting, the Lau et al. (50) results have been questioned because a nonstandard platelet function assay, the point-of-care MICROS cell counter (ABX Diagnostics, Irvine, California) and the Plateletworks test platform (Helena Laboratories, Beaumont, Texas), were used.

Others confirmed the interaction, involving not only atorvastatin but simvastatin as well (51). They also revealed that the magnitude of the interaction on clopidogrel-induced platelet inhibition decreased over time (29% reduction in platelet inhibition with combination therapy 5 h after the clopidogrel loading vs. 16% by 48 h).

Since then, other investigators have refuted the relevancy of this interaction (52–58). Retrospective analyses of data from the Plavix Reduction of New Thrombus Occurrence (PRONTO) trial, the Clopidogrel for the Reduction of Events During Observation (CREDO), and the Maximal Individual Therapy of Acute Myocardial Infarction PLUS (MITRA PLUS) registry did not find that concomitant use of statins compromised the antiplatelet effect of clopidogrel assessed both by platelet function and clinical end points (52–54). Due to their retrospective post-hoc designs, statin administration in these trials was not randomized, and the doses tested were not known (52–54).

Four prospective studies, of which three were randomized, also evaluated the possible interaction between statins and clopidogrel (55–58). Two studies found that CYP3A4-

metabolized statins did not attenuate the antiplatelet effect of clopidogrel when given as a 600-mg loading dose (55,58). In another study, neither atorvastatin nor pravastatin significantly affected the clopidogrel-induced platelet inhibition after five weeks of concomitant treatment (56). However, statin doses used were low and not representative of the current practice (55,56). In the Interaction of Atorvastatin and Clopidogrel Study (INTERACTION), the investigators performed serial measurements of 19 platelet characteristics using conventional aggregometry, rapid analyzers, and flow cytometry to demonstrate that no significant differences in the antiplatelet effect were seen between coadministration of atorvastatin or other statins and clopidogrel 4 and 24 h after loading with clopidogrel (57).

The divergent results observed in these studies may be attributed to several factors: variability in the method used to assess platelet function, small sample size (50–58), comparisons of nonequivalent statin doses (55), pooling of simvastatin and lovastatin data against atorvastatin (54,57), lack of baseline assessment of platelet aggregation before statin, and clopidogrel administration (58). These methodological limitations thus affect the interpretability of the results.

In summary, although the interaction between CYP3A4-metabolized statins and clopidogrel is possible, there is insufficient convincing data to judge the clinical consequences of this interaction at the present time. For now, clinicians should rely on landmark clinical trials that still promote the concomitant use of statins and clopidogrel in patients at high risk of coronary events (59).

Genetic implications. It is estimated that genetics can account for 20% to 95% of variability in drug disposition and efficacy (60).

POLYMORPHISMS OF THE $P2Y_{12}$ RECEPTOR. Until recently, all the mutations described for the $P2Y_{12}$ gene resulted in congenital bleeding disorders (18). Platelets of patients carrying these polymorphisms retain the capacity to change conformation when exposed to ADP, but undergo only a slight, rapidly reversible aggregation response. In most cases, the defects in the $P2Y_{12}$ gene is characterized by decreased ADP binding to platelets caused by one or two base pair deletions (each in a different location) that disrupt receptor synthesis (18). In one case, the ADP-mediated aggregation and repression of PGE_1-stimulated cAMP levels by ADP is defective despite normal ADP binding to its $P2Y_{12}$ receptor due to two missense mutations located in regions that are important for the signal transduction through the $P2Y_{12}$ receptor (61).

Lately, Fontana et al. (62) found sequence variations in the $P2Y_{12}$ gene in 98 healthy volunteers taking no medication in the previous 10 days that might explain the interindividual variability in ADP-induced platelet aggregation. Three single-nucleotide polymorphisms and a single-nucleotide insertion polymorphism in the $P2Y_{12}$ gene were discovered. H1 was defined as the major haplotype (e.g., carrying none of the four polymorphisms) and H2 as the minor haplotype (e.g., carrying all four polymorphisms). Of the 98 subjects, 74 carried no H2 allele (H1/H1), 21 showed one H2 allele (H1/H2), and 3 had two H2 alleles (H2/H2), resulting in frequencies of 86% and 14% for haplotypes H1 and H2, respectively. The H2 haplotype was associated with higher maximal platelet aggregation in response to ADP 2 μM, with median values of 34.7% in H1/H1 subjects, 67.9% in H1/H2 subjects, and 82.4% in H2/H2 subjects ($p = 0.0071$). The authors also showed that, among 184 patients with known peripheral artery disease, 30% of them had at least one H2 allele, compared with 21% of control subjects (odds ratio, 1.6; 95% confidence interval, 1.1 to 2.5; $p = 0.02$) (63). They did not mention whether patients with peripheral artery disease enrolled in the study were receiving clopidogrel or not. As stated by the authors, this small study is at risk for false-positive findings.

Thus, H2 haplotype carriers may have a greater predisposition to atherosclerotic diseases and may not respond as well to clopidogrel. It was hypothesized that an increase in the number of receptors on the platelet surface would be the most plausible explanation for the association between the H2 haplotype and the increased platelet responsiveness to ADP.

CYP3AS POLYMORPHISMS. Recently, Lau et al. (64) reiterated the contribution of CYP3A4 activity to the phenomenon of clopidogrel resistance. A significant inverse correlation was observed between platelet aggregation and CYP3A4 activity ($r = -0.6$; $p = 0.003$) as measured by the erythromycin breath test in healthy volunteers. The investigators also demonstrated that by enhancing the CYP3A4 activity with rifampin in 10 healthy volunteers, 3 initial nonresponders (platelet inhibition $<10\%$) and one low responder (platelet inhibition between 10% to 29%) to clopidogrel exhibited enhanced platelet inhibition that then met the definition for a clopidogrel responder (platelet inhibition $\geq 30\%$).

Because CYP3A4 and CYP3A5 have overlapping substrate specificity, it is difficult to segregate the relative contributions of the two enzymes to CYP3A-mediated metabolism (65); CYP3As expression and activity vary among individuals even in the absence of drug-mediated inhibition or induction. It is estimated that most of this variability is caused by individual genetic makeup (66). This interindividual variability in CYP3As function may have a profound effect in the efficacy of clopidogrel but has not been assessed in any study. Currently, more than 30 single-nucleotide polymorphisms of CYP3A4 have been identified (65). These coding variants may contribute to, but are not likely to be, the major cause of interindividual differences in CYP3A-dependent metabolism, because of the low allele frequencies and limited alterations in enzyme expression or catalytic function. So far, over 10 single-nucleotide polymorphisms have been identified in the CYP3A5 gene (67); CYP3A5*3 is the most common and

functionally important variant across all ethnic population studied. This variant confers low CYP3A5 protein expression (67).

CONCLUSIONS

Current available data show that about 4% to 30% of patients treated with conventional doses of clopidogrel do not display adequate antiplatelet response. Clopidogrel resistance is a term widely used but not clearly defined. So far, it has been used to reflect failure of clopidogrel to achieve its platelet inhibition effect. Preliminary results seem to indicate that low antiplatelet effect of clopidogrel may lead to higher risk of developing cardiovascular events. However, the optimal level of clopidogrel-induced platelet inhibition that will correlate quantitatively with clopidogrel's ability to prevent atherothrombotic events is still lacking.

The interpatient variability in clopidogrel response is multifactorial. It can be due to extrinsic or intrinsic mechanisms such as drug-drug interactions involving CYP3A4, or genetic polymorphisms of the $P2Y_{12}$ receptor and CYP3As.

In the literature, the terms of *clopidogrel resistance*, *nonresponse*, and *low response to clopidogrel* are used synonymously, which may confuse readers. Because the response to clopidogrel has been mostly evaluated by platelet function tests, these terms can be considered as interchangeable as they reflect failure of clopidogrel to achieve its expected antiplatelet effect. For clarity, we propose using the term clopidogrel resistance.

Presently, it is impossible to predict which patient will be resistant to clopidogrel. Furthermore, because there is currently no single and validated platelet function assay to measure clopidogrel antiplatelet effect, it is not justified to routinely look for clopidogrel resistance in the clinical setting. Although, no proven therapy is currently available to overcome low responsiveness to clopidogrel, recent clinical data favor use of an increased loading dose of clopidogrel in patients undergoing PCI. Factors that may modulate individual response to clopidogrel should be better evaluated in larger controlled clinical trials. This may also help tailor therapy with future antiplatelet alternatives to aspirin and clopidogrel.

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