Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 49, No. 14, 2007
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2006.11.044

STATE-OF-THE-ART PAPER

Variability in Individual Responsiveness to Clopidogrel

Clinical Implications, Management, and Future Perspectives

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Antiplatelet therapy is the cornerstone of treatment for patients with acute coronary syndromes and/or undergoing percutaneous coronary interventions. Clopidogrel, in combination with aspirin, is currently the antiplatelet treatment of choice for prevention of stent thrombosis, and clinical trials have shown that, in high-risk patients, prolonged dual antiplatelet treatment is more effective than aspirin alone in preventing major cardiovascular events. However, despite the use of clopidogrel, a considerable number of patients continue to have cardiovascular events. Numerous in vitro studies have shown that individual responsiveness to clopidogrel is not uniform in all patients and is subject to inter- and intraindividual variability. Notably, there is a growing degree of evidence that recurrence of ischemic complications may be attributed to poor response to clopidogrel. The mechanisms leading to poor clopidogrel effects are not fully elucidated and are likely multifactorial. Although the gold standard definition to assess antiplatelet drug response has not been fully established, there is sufficient evidence to support that persistence of enhanced platelet reactivity despite the use of clopidogrel is a clinically relevant entity. This paper reviews the impact of individual response variability to clopidogrel on clinical outcomes and current and future directions for its management. (J Am Coll Cardiol 2007;49:1505–16) © 2007 by the American College of Cardiology Foundation

Platelets play a key role in the pathophysiology of thrombosis after plaque rupture (1). Plaque rupture occurs spontaneously in patients with acute coronary syndromes (ACS), or may be iatrogenically induced in patients undergoing percutaneous coronary interventions (PCI). Among the multiple mediators of platelet activation, adenosine diphosphate (ADP) plays a pivotal role. Adenosine diphosphate binds to several receptors on the platelet membrane (2). Thienopyridines are irreversible inhibitors of the ADP $P2Y_{12}$ receptor. Ticlopidine is a first-generation thienopyridine, which, in combination with aspirin, has shown to be beneficial and superior to oral anticoagulants in preventing thrombotic complications after coronary stenting (3–6). Today clopidogrel, a second-generation thienopyridine with similar efficacy, has largely replaced ticlopidine due to its better tolerability profiles (7) and is currently the antiplatelet treatment of choice for prevention of stent thrombosis (8). In addition, in patients undergoing PCI, prolonged dual antiplatelet therapy has been associated with better long-term clinical outcomes (9,10). The long-term clinical benefit associated with dual antiplatelet therapy has been observed overall in patients with unstable angina and non–ST-segment elevation myocardial infarction (STEMI) independent of coronary revascularization (11). More recently, the spectrum of clinical benefit of clopidogrel has also been extended to patients with STEMI (12,13).

Despite the unambiguous clinical benefit achieved with the adjunct of clopidogrel in ACS/PCI patients, a considerable number of patients continue to have cardiovascular events. This has been, in part, attributed to the fact than some patients may have poor clopidogrel-induced antiplatelet effects. These patients, in fact, despite treatment with clopidogrel, persist with enhanced platelet reactivity, which is pivotal for the development of atherothrombotic complications (1). Although the mechanisms leading to poor clopidogrel effects are not fully elucidated and the best definition to assess antiplatelet drug response has not been fully established, there is sufficient evidence to support that persistence of enhanced platelet reactivity despite the use of clopidogrel is a clinically relevant entity. This paper reviews the impact of individual response variability to clopidogrel

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Manuscript received August 9, 2006; revised manuscript received November 13, 2006, accepted November 28, 2006.

Abbreviations and Acronyms

ACS = acute coronary syndrome
ADP = adenosine diphosphate
ATP = adenosine triphosphate
cAMP = cyclic adenosine monophosphate
CYP = cytochrome P450
GP = glycoprotein
GTP = guanosine triphosphate
LTA = light transmittance aggregometry
MFI = median fluorescence intensity
NSTE-ACS = non–ST-segment elevation acute coronary syndrome
PCI = percutaneous coronary intervention
PGE_1 = prostaglandin E_1
STEMI = ST-segment elevation myocardial infarction
VASP-P = vasodilator-stimulated phosphoprotein-phosphorylation

on clinical outcomes and current and future directions for its management.

ADP Receptors and Mechanism of Action of Clopidogrel

Adenosine diphosphate is one of the most important mediators of both physiological hemostasis and thrombosis (2). After platelet activation, ADP is not only released from its intracellular storage granules but also further activates platelets, amplifying this process. There are 2 main purinergic receptor types in the membrane: the guanosine triphosphate (GTP)-coupled protein receptors, known as G-protein binding sites, and the ligand-gated ion channel (2). The latter receptor is designated $P2X_1$ and the former is designated as P2Y, and each play a specific and complimentary role in platelet activation and aggregation (Fig. 1).

The $P2X_1$ utilizes adenosine triphosphate (ATP) as an agonist and mediates extracellular calcium influx leading to alteration in platelet shape. There are 2 known P2Y receptors: $P2Y_1$ and $P2Y_{12}$, which utilize ADP as an agonist. Activation of the $P2Y_1$ receptor leads to a series of signaling events that initiate a weak and transient phase of platelet aggregation. In particular, $P2Y_1$ is coupled to a G_q protein, and its intracellular signaling pathways involve activation of phospholipase C resulting in diacylglycerol and inositol triphosphate production. Diacylglycerol activates protein kinase C, which leads to phosphorylation of myosin light chain kinase and granule secretion; inositol triphosphate leads to mobilization of intracellular calcium. The $P2Y_1$ receptor is coupled to another G-protein, G_{12}, which activates the "Rho" protein and is believed to lead to change in platelet shape. Activation of the $P2Y_{12}$ receptor leads to a complex series of intracellular signaling events that result in activation of the glycoprotein (GP) IIb/IIIa receptor, granule release, amplification of platelet aggregation, and stabilization of the platelet aggregate. The $P2Y_{12}$ receptor is coupled to a G_i protein and its intracellular signaling pathways involve activation of phosphoinositide-3-kinase and inhibition of adenylyl cyclase. Phosphoinositide-3-kinase activation leads to GP IIb/IIIa activation through activation of a serine-threonine protein kinase B and of Rap1b GTP-binding proteins. Inhibition of adenylyl cyclase decreases cyclic adenosine monophosphate (cAMP) levels. Reduction of cAMP levels influences the activity of cAMP-dependent protein kinases that, in turn, reduce cAMP-mediated phosphorylation (P) of vasodilator-stimulated phosphoprotein (VASP) and eliminate its protective effect on GP IIb/IIIa receptor activation (Fig. 1).

Clopidogrel selectively and irreversibly inhibits the $P2Y_{12}$ receptor (14). Clopidogrel is an inactive pro-drug that requires oxidation by the hepatic cytochrome P450 (CYP) system to generate an active metabolite. However, ~85% of the pro-drug is hydrolyzed by esterases in the blood to an inactive carboxylic acid derivative, and only ~15% of the pro-drug is metabolized by the CYP system in the liver to generate an active metabolite. In particular, the thiophene ring of clopidogrel is oxidized to form an intermediate metabolite (2-oxo-clopidogrel), which is further oxidized, resulting in the opening of the thiophene ring and the formation of a carboxyl and thiol group. The reactive thiol group of the active metabolite of clopidogrel forms a disulfide bridge between 1 or more cysteine residues of the $P2Y_{12}$ receptor, resulting in its irreversible blockade for the life span of the platelet. Thus, $P2Y_{12}$ receptor blockade acts early in the cascade of events leading to the formation of the platelet thrombus and effectively inhibits platelet aggregation. In fact, platelet $P2Y_{12}$ blockade prevents platelet degranulation and the release reaction, which elaborates prothrombotic and inflammatory mediators from the platelet, and also inhibits the transformation of the GP IIb/IIIa receptor to the form that binds fibrinogen and links platelets (Fig. 1).

Platelet Function Testing

Several methods have been used to assess clopidogrel-induced antiplatelet effects (15) (Table 1). However, none of these tests have been fully standardized or fully agreed upon to measure clopidogrel responsiveness. Turbidometric light transmittance aggregometry (LTA) using ADP as an agonist is currently considered the gold standard technique to assess clopidogrel response. Although most investigations have used this technique to assess clopidogrel response variability, several definitions of poor clopidogrel response have been adopted. In addition, LTA may be subject to several methodological variables that may also lead to variances in the prevalence of poor responders. These may include the dose of agonist (5 or 20 μmol/l), the nature of the anticoagulant (citrate or hirudin/PPACK), and the LTA value (maximal or late platelet aggregation), among many other variables, used for the assessment (16,17). In addition to these methodological variables, LTA is time-consuming, technically demanding, and not available in most centers, limiting its broad scale application.

Defining clopidogrel-induced antiplatelet effects using LTA is also confounded by the fact that although clopidogrel prevents ADP from inducing platelet activation through inhibition of the $P2Y_{12}$ receptor, the other ADP receptor subtypes can still be activated and contribute to the

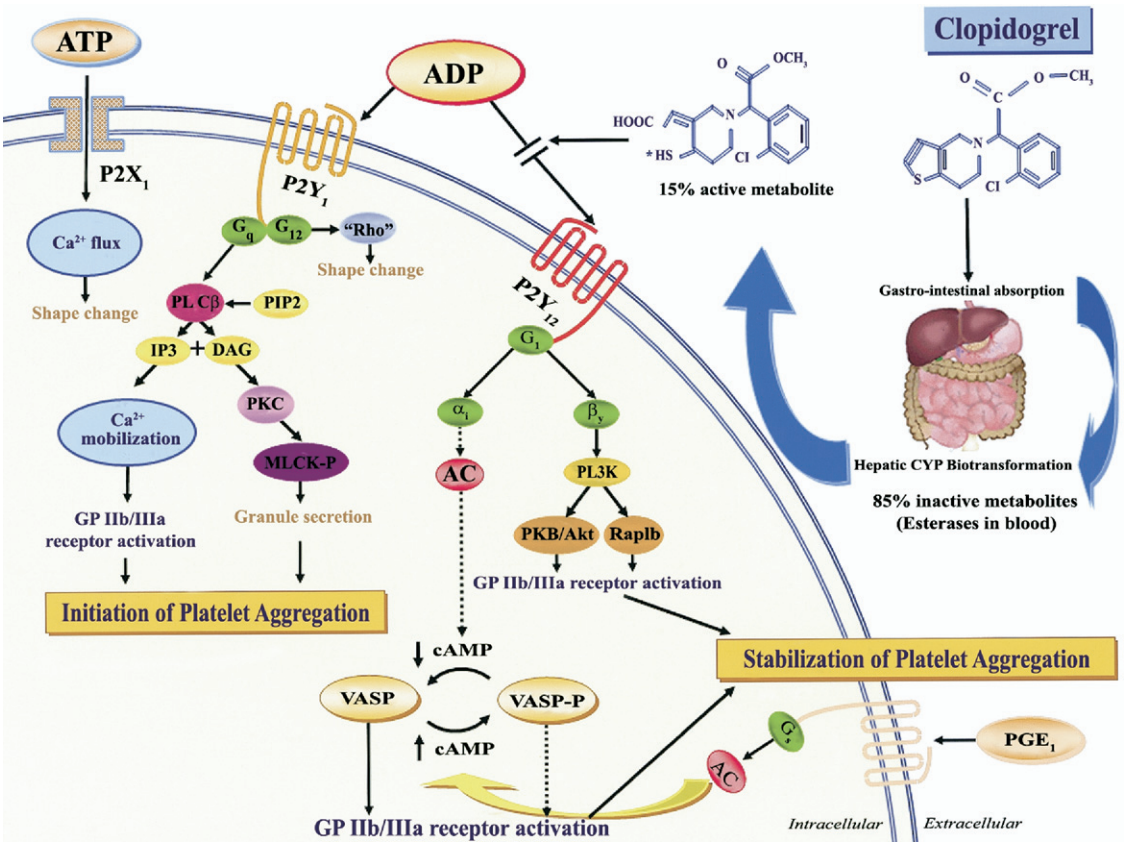

Figure 1 **P2 Receptors and Mechanism of Action of Clopidogrel**

Clopidogrel is a pro-drug administered orally. Approximately 85% of the pro-drug is hydrolyzed by esterases in the blood to an inactive carboxylic acid derivative, and only 15% of the pro-drug is metabolized by the cytochrome P450 (CYP) system in the liver to generate an active metabolite. The active metabolite irreversibly inhibits the adenosine diphosphate (ADP) $P2Y_{12}$ receptor. Activation of the $P2X_1$ and $P2Y_1$ receptors leads to alteration in shape and initiates a weak and transient phase of platelet aggregation. The $P2X_1$ mediates extracellular calcium influx and utilizes adenosine triphosphate (ATP) as an agonist. The binding of ADP to the G_q-coupled $P2Y_1$ receptor leads to activation of phospholipase C (PLC), which generates diacylglycerol (DAG) and inositol triphosphate (IP3) from phosphatidylinositol bisphosphate (PIP2). Diacylglycerol activates protein kinase C (PKC) leading to phosphorylation of myosin light chain kinase (MLCK-P); IP3 leads to mobilization of intracellular calcium. The $P2Y_1$ receptor is coupled to another G-protein, G_{12}, which activates the "Rho" protein and is believed to lead to change in platelet shape. The binding of ADP to the G_i-coupled $P2Y_{12}$ receptor liberates the G_i protein subunits α_i and β_γ and results in stabilization of platelet aggregation. The α_i subunit leads to inhibition of adenylyl cyclase (AC), which reduces cyclic adenosine monophosphate (cAMP) levels. This, in turn, diminishes cAMP-mediated phosphorylation of vasodilator-stimulated phosphoprotein (VASP) (VASP-P). The status of VASP-P modulates glycoprotein (GP) IIb/IIIa receptor activation. The subunit β_γ activates the phosphatidylinositol 3-kinase (PI3K), which leads to GP IIb/IIIa receptor activation through activation of a serine-threonine protein kinase B (PKB/Akt) and of Rap1b GTP binding proteins. Prostaglandin E_1 (PGE_1) activates AC, which increases cAMP levels and status of VASP-P. **Solid arrows** = activation; **dotted arrows** = inhibition.

final degree of platelet aggregation (2). This has led to the development of assays more specific to the $P2Y_{12}$ pathway. Flow cytometric assessment of VASP-P is a marker of $P2Y_{12}$ receptor reactivity and, thus, clopidogrel-induced inhibition (18). It uses 2 different agonists: prostaglandin E_1 (PGE_1) and ADP. Prostaglandin E_1 increases VASP-P levels by stimulation of adenylate cyclase. Binding of ADP to $P2Y_{12}$ leads to G_i-coupled inhibition of adenylate cyclase. Therefore, the addition of ADP to PGE_1-stimulated platelets reduces PGE_1-induced VASP-P levels. If $P2Y_{12}$ receptors are successfully inhibited by clopidogrel, addition of ADP will not reduce the PGE_1-stimulated VASP-P levels. Assessing median fluorescence intensity (MFI) of VASP-P levels using this approach has allowed the definition of the $P2Y_{12}$ reactivity ratio, which is calculated as follows: ([MFI PGE_1] − [MFI PGE_1 + ADP]/[MFI PGE_1]) × 100%. A reduced $P2Y_{12}$ reactivity ratio is indicative of more enhanced clopidogrel-induced inhibition. Another advantage of the VASP assay is that samples can be fixed and shipped to central core laboratories for its assessment. This allows to overcome the technical limitations that many centers may have in performing these highly specific assays. Nevertheless, this does not overcome the need for rapid, widely available, and reliable bedside measures of platelet aggregation.

The VerifyNow $P2Y_{12}$ point-of-care assay (Accumetrics, San Diego, California) has been recently approved by the Food and Drug Administration. This is a user-friendly system that rapidly measures in whole blood the rate and extent of platelet aggregation (19). In particular, it performs

JACC Vol. 49, No. 14, 2007
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Table 1 Platelet Function Tests Assessing Adenosine Diphosphate Receptor Antagonism

Platelet aggregation
Turbidometric light transmittance aggregometry
Impedance platelet aggregation
Flow cytometry
GP IIb/IIIa receptor activation
P-selectin expression
Leucocyte-platelet aggregates
Vasodilator-associated stimulated phosphoprotein phosphorylation
Point-of-care
VerifyNow $P2Y_{12}$ assay
Thromboelastagraph Platelet Mapping System
Plateletworks
Impact cone and plate(let) analyzer

GP = glycoprotein.

a turbidimetric measurement of agglutination of platelets to fibrinogen-coated micro-beads. This assay applies a similar principle to that used by the VASP platelet assay in order to specifically evaluate inhibition of the $P2Y_{12}$ pathway as it uses a combination of ADP and PGE_1 as agonists in order to increase the specificity of the test. In addition, a separate well containing thrombin receptor activating peptide (TRAP) provides a baseline platelet function assessment, allowing the measurement of the degree of platelet inhibition in patients on clopidogrel without having to wean the patient off clopidogrel. Given their broad availability and simple-to-use characteristics, similar assays are more practical in the clinical setting and have a promising role in the future for guiding antiplatelet treatment. Ongoing studies are currently evaluating the clinical risk of patients based on the results of such point-of-care assays.

Definition of Clopidogrel Responsiveness

Standardized definitions to define individual responsiveness to clopidogrel are still lacking. This is due not only to the numerous assays currently available to assess clopidogrel-induced antiplatelet effects but also to the methodological variability within each technique (i.e., LTA). Light transmittance aggregometry has been most extensively evaluated to define clopidogrel responsiveness, and several definitions to define clopidogrel responsiveness have been used. Earlier studies have defined clopidogrel responsiveness according to the absolute differences between pre- and post-treatment platelet reactivity (20); other studies have defined clopidogrel responsiveness according to the degree of inhibition of platelet aggregation or IPA, defined as the percent decrease in aggregation values obtained at baseline and after treatment (21). These studies have shown clopidogrel-induced antiplatelet effect to be highly variable and that a considerable number of patients may have poor or no antiplatelet effects. Using an arbitrary cutoff value of <10% with the respective definitions, these ex-vivo platelet function studies have thus led to define these individuals with poor antiplatelet effects as "clopidogrel resistant" or "non-responders." However, subsequent investigations have empirically used different doses of agonists, different cutoff values, and different assays to define clopidogrel-induced antiplatelet effects resulting in a highly variable reported prevalence of poor clopidogrel responders (22,23).

Use of different nomenclature to define individuals with ineffective clopidogrel platelet inhibition, such as "low-responder," "hypo-responder," "semi-responder," "suboptimal responder" among others and in addition to the ones described in the preceding text, has also further compounded the confusion on this topic. However, the increasing knowledge on the clinical impact of interindividual variability of clopidogrel-induced antiplatelet effects has allowed progress in our current definitions. In fact, previous definitions of clopidogrel response, which imply knowledge of baseline platelet function for its assessment, overestimate ischemic risk compared with post-treatment values of platelet reactivity (24). Given the better prognostic implications of post-treatment platelet reactivity, current investigations are now aiming to establish therapeutic thresholds to define optimal $P2Y_{12}$ inhibition in clopidogrel-treated patients. Use of post-treatment platelet reactivity values as a measure of effectiveness of clopidogrel effects is in line with how other biological variables and their response to treatment are quantified. Accordingly, as per other biological processes, clopidogrel responsiveness should not be considered in a dichotomous way (25), but as a continuous and variable parameter (Fig. 2). In the present review, we will allude to

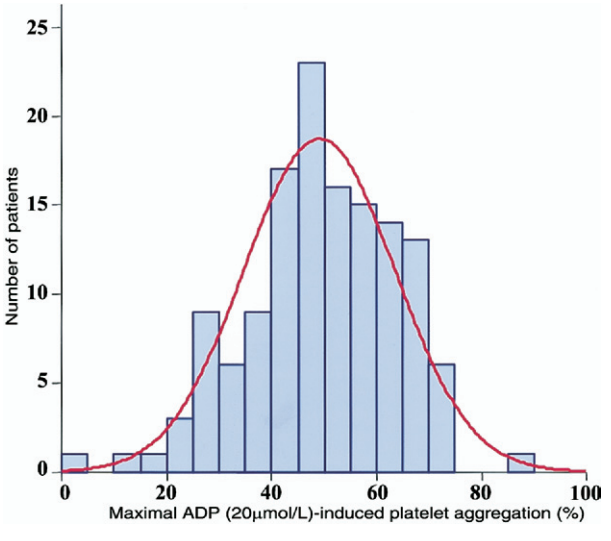

Figure 2 Interindividual Variability in Platelet Aggregation

Platelet aggregation profile (maximal 20 μmol/l ADP-induced platelet aggregation using light transmittance aggregometry) in patients (n = 135) in a steady state phase (>1 month) of combined aspirin (100 mg/day) and clopidogrel (75 mg/day) therapy. Heterogeneous antiplatelet effects are observed in the overall patient population as depicted by the normal bell-shaped distribution of platelet aggregation. ADP = adenosine diphosphate (D.J. Angiolillo, unpublished data, 2006).

the term "clopidogrel response variability" to indicate such interindividual variation in antiplatelet effects.

Mechanisms Leading to Clopidogrel Response Variability

The mechanisms leading to variability in clopidogrel responsiveness are not fully elucidated and, similarly to aspirin, are like multifactorial (Fig. 3). High pre-treatment platelet reactivity may contribute to reduced clopidogrel-induced antiplatelet effects (20,22). Increased baseline platelet reactivity may be more commonly observed in specific clinical scenarios such as ACS, increased body mass index, and diabetes mellitus, in particular insulin-dependent diabetes mellitus (26–29). Therefore, patient selection may influence the prevalence of poor clopidogrel responders in a given study. Other clinical factors that may lead to reduced clopidogrel effects include lack of drug prescription, poor compliance, and inappropriate dosing.

Differences in individual absorption of clopidogrel as well as levels of its active metabolite may also lead to clopidogrel response variability (30). Drugs that are substrates or inhibit the CYP isoenzyme 3A4 can potentially interfere with the conversion of clopidogrel into its active metabolite, leading to reduced antiplatelet effects (31). In particular, studies have shown that lipophilic statins, such as atorvastatin and simvastatin, which require CYP3A4 metabolization, hamper clopidogrel-induced antiplatelet effects (31,32). However, these data are quite controversial as larger studies have shown the lack of any interaction between lipophilic statins and clopidogrel (33–36). In addition, most studies do not show any negative clinical interaction with coadministration of these drugs (37). Independent of these potential drug-drug interactions, baseline metabolic activity of this enzyme may also contribute to variability of clopidogrel-induced antiplatelet effects (38). In fact, individuals with low baseline CYP3A4 activity, which decreases clopidogrel activation, have been shown to have suboptimal clopidogrel responsiveness (38).

The metabolic activity of the CYP3A4 enzyme is under genetic control and varies considerably among individuals (39,40). Genetic polymorphisms of this and other CYP enzymes have been shown to modulate individual responsiveness to clopidogrel (39,40). The impact of other genetic polymorphisms on clopidogrel response has also been evaluated. A minor haplotype of the $P2Y_{12}$ receptor was found to be associated with increased platelet reactivity in non-medicated healthy volunteers (41). However, these findings could not be duplicated by several authors studying patients with coronary artery disease treated with clopidogrel (42–44). Small sample size studies have shown a potential role of genetic polymorphisms of the GP IIb/IIIa receptor on modulation of clopidogrel response in the acute phase of treatment (45,46). However, this has not been confirmed by others (44), and our group failed to replicate our initial findings performed in the acute phase of clopidogrel treatment in patients on chronic clopidogrel therapy (47).

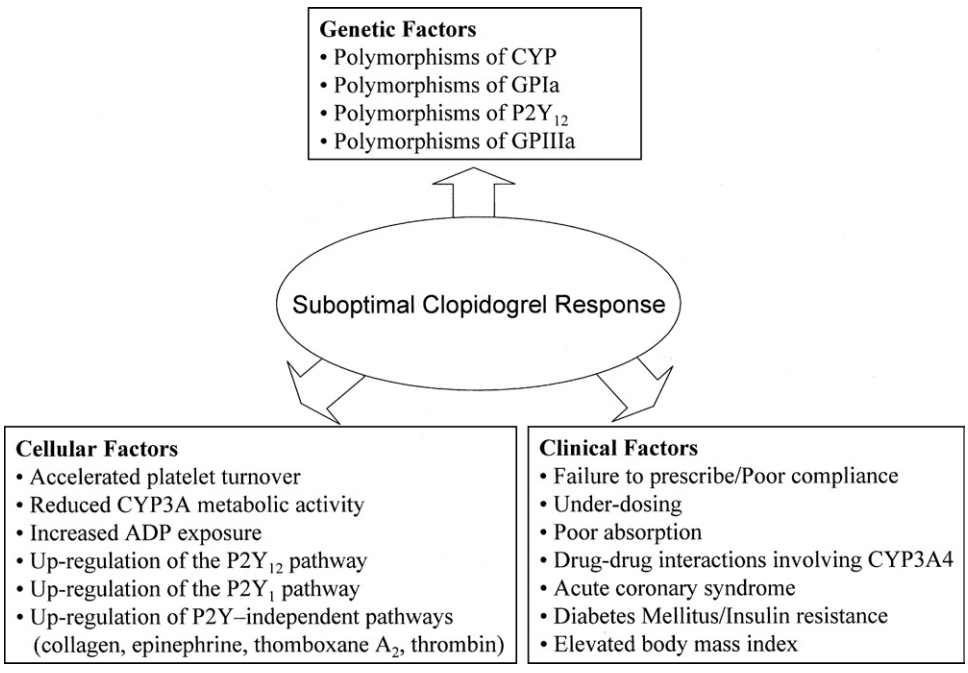

Figure 3 Proposed Mechanisms Leading to Variability in Individual Responsiveness to Clopidogrel

ADP = adenosine diphosphate; CYP = cytochrome P450; GP = glycoprotein.

Overall, these findings are likely related to the fact that an active metabolite, and not clopidogrel per se, is responsible for inhibition of the $P2Y_{12}$ receptor, suggesting therefore that an upstream target within clopidogrel's metabolic pathway (i.e., CYP) has a more important modulating role of its downstream antiplatelet effects (39,48,49). Recent reports have also suggested that polymorphisms of other targets not directly involved in clopidogrel metabolism may be involved in response variability. These include platelet membrane receptors, such as GP Ia, which are pivotal for aggregatory responses (50,51).

Other mechanisms leading to variability in clopidogrel responsiveness may include increase exposure to ADP, up-regulation of the $P2Y_{12}$ pathway, and up-regulation of $P2Y_{12}$-independent pathways (26,29,52). The latter includes enhanced ADP-induced platelet aggregation through the $P2Y_1$ pathway as well as up-regulation of pathways independent of ADP, such as collagen, epinephrine, thromboxane A_2, and especially thrombin.

Clinical Implications of Individual Response Variability to Clopidogrel

Several studies have shown the clinical implications of individual response variability to clopidogrel. Clinical outcomes include stent thrombosis, post-stent ischemic events, and periprocedural myocardial infarction. These studies have been performed in different subgroups of patients undergoing PCI, including patients with STEMI, non–ST-segment elevation (NSTE)-ACS, and patients undergoing elective PCI.

Stent thrombosis. The first study to hypothesize the clinical implications of clopidogrel responsiveness was reported by Muller et al. (21). In this study, LTA was performed in a cohort of 105 patients undergoing PCI in which 2 incidents of subacute stent thrombosis occurred, and both patients were clopidogrel non-responders. Barragan et al. (53) carried out a prospective evaluation using a VASP assay and observed that patients experiencing subacute stent thrombosis had significantly enhanced platelet reactivity. Ajzenberg et al. (54) demonstrated that patients with subacute stent thrombosis have increased shear-induced platelet aggregation compared with control subjects receiving dual antiplatelet therapy and with normal subjects receiving no antiplatelet therapy. The CREST (Clopidogrel Effect on Platelet REactivity in Patients With Stent Thrombosis) study showed that high post-treatment platelet reactivity, assessed by LTA, and incomplete $P2Y_{12}$ receptor inhibition, assessed by VASP, were risk factors for subacute stent thrombosis (55).

Post-stent ischemic events and periprocedural myocardial infarction. The first study suggesting the impact of clopidogrel-induced antiplatelet effects on post-stent ischemic events was from Matetzky et al. (56). In this study, patients (n = 60) with STEMI undergoing primary PCI were stratified into 4 quartiles according to the percentage reduction of ADP-induced platelet aggregation using LTA. Whereas 40% of patients in the first quartile sustained a recurrent cardiovascular event (STEMI, ACS, subacute stent thrombosis, and acute peripheral arterial occlusion) during 6-month follow-up, only 1 patient (6.7%) in the second quartile and none in the third and fourth quartiles suffered a cardiovascular event ($p = 0.007$). Cuisset et al. (57) studied a series of 106 NSTE-ACS patients treated with PCI in whom LTA was assessed at the time of intervention; in this series 12 ischemic events occurred at 1-month follow-up, of which 9 occurred in the highest aggregation quartile and 3 in the second highest quartile. Recently, the same authors also showed that in NSTE-ACS patients (n = 292) undergoing coronary stenting, the use of a 600-mg clopidogrel loading dose reduced the number of patients with high post-treatment platelet reactivity compared with a standard 300-mg loading dose regimen, which also resulted in improved clinical outcomes (58).

The largest prospective study supporting the prognostic implications of platelet function measures is the EXCELSIOR (Impact of Extent of Clopidogrel-Induced Platelet Inhibition During Elective Stent Implantation on Clinical Event Rate) study (59). In this study, a cohort of relatively low-risk patients (n = 802) undergoing elective coronary stenting pre-treated at least 2 h before intervention with a 600-mg loading dose of clopidogrel was evaluated. Patients showing higher degrees of "late" 5 μmol/l ADP-induced platelet aggregation assessed by LTA immediately "before" intervention (those in the 2 upper quartiles) suffered more frequently 30-day major adverse cardiac events, which occurred in 15 patients. Notably, platelet aggregation above the absolute median value of the study population (14% of aggregation) carried a 6.7-fold risk of events. Furthermore, on multivariate logistic regression models (including all potential confounders such as baseline platelet aggregation and time from clopidogrel loading to intervention) platelet aggregation emerged as an independent predictor of early clinical outcome.

The CLEAR PLATELETS (Clopidogrel Loading with Eptifibatide to Arrest PLATELET reactivity) and CLEAR PLATELETS Ib studies showed that in patients (n = 120) undergoing elective PCI, a 600-mg loading dose of clopidogrel was associated with superior early platelet inhibition compared with a 300-mg loading dose (60,61). This enhanced platelet inhibition was sustained over 24 h and was accompanied by a decrease in release of myocardial necrosis and inflammatory markers. Gurbel et al. (62) also showed in the PREPARE POST-STENTING (Platelet REactivity in Patients And Recurrent Events POST-STENTING) study, that higher degrees of "maximal" 20 μmol/l ADP-induced platelet aggregation assessed by LTA "at discharge" was associated with ischemic events ($63 \pm 12\%$ vs. $56 \pm 15\%$, $p = 0.02$) at 6 months in patients (n = 192) undergoing non-emergent stenting. All patients were treated with a loading dose of clopidogrel (300 or 600 mg) "after" intervention. Notably, ~90% of patients with events had post-treatment platelet reactivity values above 50%. In line with

the prognostic implications of high post-treatment platelet reactivity after acute clopidogrel administration, even patients on chronic clopidogrel therapy undergoing non-emergent PCI, those who exhibit high on-treatment ADP-induced platelet aggregation are at increased risk for post-procedural ischemic events up to 6 months (63).

Lev et al. (64) evaluated response to clopidogrel using LTA in aspirin-resistant and -sensitive patients (n = 150) undergoing elective PCI. Aspirin-resistant patients had lower response to clopidogrel than aspirin-sensitive patients and ~50% of aspirin-resistant patients were also resistant to clopidogrel. Elevation of cardiac enzymes after stenting occurred more frequently in aspirin-resistant (12.7%) versus aspirin-sensitive patients and in clopidogrel-resistant (24%) versus clopidogrel-sensitive patients. Patients with dual drug resistance had higher incidence of cardiac enzyme elevation than the respective sensitive patients (44.4% vs. 15.8%; p = 0.05).

Geisler et al. (65) assessed responsiveness to clopidogrel using LTA in a total of 379 consecutive patients (stable angina n = 206 and ACS n = 173) undergoing PCI treated with a 600-mg loading dose. At 3-month follow-up, the primary outcome of a combined major cardiovascular event including nonfatal myocardial infarction, non-fatal ischemic stroke, or cardiovascular death showed that low responders (5.8%) had a significantly higher risk of major cardiovascular events compared with patients who adequately responded to clopidogrel (22.7% vs. 5.6%; odds ratio 4.9; p = 0.004). After adjustment for other factors influencing cardiovascular outcome, low response to clopidogrel and severe left ventricular dysfunction were independently associated with a major cardiovascular event within 3 months (hazard ratio for low response to clopidogrel 3.71; p = 0.037).

Management of Patients With Poor Clopidogrel Response

Variability in clopidogrel-induced antiplatelet effects has become an emerging clinical entity with potentially severe consequences (66). Therefore, it becomes imperative to question how a clinician can effectively manage this phenomenon. Unfortunately, not only the definition but also how to treat these patients remains undefined. An initial approach would be to correct the clinical factors that may be leading to poor responsiveness. Importantly, physicians must ensure proper patient compliance. Albeit controversial, decreasing drug–drug interactions (e.g., CYP3A4 metabolizing statins) may potentially optimize response. Since abnormal lipid, glucose, and blood pressure levels may lead to abnormalities of the platelet plasmatic membrane and platelet dysfunction, their control may enable better response to antiplatelet agents.

Several studies have focused on the impact of the loading dose of clopidogrel utilized in patients undergoing PCI and have clearly defined underdosing as a pivotal cause to poor responsiveness. In fact, although a 300-mg loading dose is the standard loading dose regimen to be given in patients undergoing PCI, several functional studies have shown that a 600-mg loading dose leads to an earlier, stronger, and more sustained inhibition of platelet function (23,67). Using a 600-mg loading dose regimen, full antiplatelet effects are achieved after 2 h (68). Furthermore, a high loading dose regimen may also prevent the reduction of platelet inhibition by concomitant use of statins metabolized by CYP3A4 (34,36). The ARMYDA-2 (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-2) study showed the benefit of pre-treatment with a 600-mg loading dose when compared with 300-mg in reducing periprocedural myocardial infarction in patients undergoing PCI, without any increase in bleeding hazards (69). Similar outcome results have been recently replicated by the Cuisset et al. (58) study, in which improved clinical outcomes were also corroborated by better platelet inhibition with a high loading dose regimen.

Despite these studies supporting the functional and clinical impact of a high loading dose regimen and its broad application in daily clinical practice by most interventionalists, these are overall small sample size studies, and the use of a 600-mg loading dose regimen has still not been cleared by the Food and Drug Administration. The ongoing CURRENT/OASIS-7 (Clopidogrel optimal loading dose Usage to Reduce Recurrent EveNTs/Optimal Antiplatelet Strategy for InterventionS) trial will evaluate whether high-dose clopidogrel achieves better clinical outcomes than standard dose in ~14,000 NSTE-ACS patients undergoing PCI. Patients randomized to the high dose will receive a 600-mg loading dose then 150-mg daily maintenance dose from day 2 to day 7; patients randomized to the standard dose will receive a 300-mg loading dose then 75-mg daily maintenance dose from day 2 to day 7; from day 8 to day 30, all patients will receive clopidogrel 75 mg daily. In addition, all patients will get randomized to receive aspirin low dose (75 to 100 mg) or high dose (300 to 325 mg); regardless of randomized allocation to high- or low-dose aspirin, all patients will receive aspirin ≥300 mg on day 1. Other ongoing studies evaluating a 600-mg clopidogrel loading dose regimen include ARMYDA-4, which will determine the clinical benefit of a further loading dose of 600-mg clopidogrel pre-PCI in patients already on chronic treatment, and ARMYDA-5, which will assess clinical outcomes of patients undergoing PCI with a pre-loading strategy of 600-mg clopidogrel 4 to 8 h before PCI versus in-lab administration of a 600-mg loading dose after coronary angiography, immediately pre-PCI. The latter will address a highly debated issue in daily clinical practice, which is the problem of knowing coronary anatomy before giving the drug.

The utility of increasing the loading dose of clopidogrel to 900 mg has been recently evaluated in the ALBION (Assessment of the Best Loading Dose of Clopidogrel to Blunt Platelet Activation, Inflammation, and Ongoing Necrosis) and the ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choose Between 3 High

Oral Doses for Immediate Clopidogrel Effect) trials (70,71). Although a high loading dose regimen (600 mg and 900 mg) showed a greater and faster degree of platelet inhibition compared with a 300-mg loading dose, the differences observed between a 600- and 900-mg loading regimen were less remarkable. Therefore, although clopidogrel response is dose-dependent, there is a threshold to its platelet inhibitory effects when certain doses are reached. This supports the need for the adjunctive use of a GP IIb/IIIa inhibitor in patients with poor clopidogrel response and in whom more potent platelet inhibition is warranted (60,72). In line with this observation, the CLEAR-PLATELETS study showed that the use of a GP IIb/IIIa inhibitor produces superior platelet inhibition resulting in lower myocardial necrosis compared with high (600-mg) or standard (300-mg) clopidogrel loading dose alone (60).

Recently, it was shown that the administration of a 600-mg loading dose in patients already on chronic clopidogrel therapy results in an additional significant increase in inhibition of ADP-induced platelet aggregation, suggesting that the current recommended maintenance dose of clopidogrel may be insufficient in producing optimal platelet inhibition (73). The currently used maintenance dose for chronic clopidogrel therapy (75 mg/day) was chosen because a degree of platelet inhibition is reached similar to that achieved with ticlopidine 250 mg twice daily (74). Therefore, it has been suggested that increasing the maintenance dose to 150 mg/day may improve individual responsiveness to clopidogrel in selected patient populations. The ISAR-CHOICE-2 (Intracoronary Stenting and Antithrombotic Regimen: Choose a High Oral maintenance dose for Intensified Clopidogrel Effect) showed that in an unselected cohort of patients a 150-mg maintenance dose resulted in enhanced platelet inhibition compared with a standard 75-mg maintenance dose regimen 1 month after undergoing low-risk PCI (75). The OPTIMUS (Optimizing anti-Platelet Therapy In diabetes MellitUS) study selectively studied diabetes mellitus patients with high post-treatment platelet reactivity while in their chronic phase of treatment (76). In these patients, although a 150-mg clopidogrel maintenance dose resulted in marked platelet inhibition of numerous platelet function measures compared with a 75-mg dose, a considerable number of patients still remained above the therapeutic threshold of post-treatment platelet reactivity used in this study, suggesting the need for more potent $P2Y_{12}$ inhibitors or alternative antithombotic regimens in these high-risk patients. However, studies sufficiently powered to assess safety and clinical impact are warranted before using platelet function testing and adjustment of antithrombotic drug regimens in clinical practice. Current guidelines state (class IIb indication with a level of evidence C) that only patients in whom stent thrombosis may be catastrophic or lethal (unprotected left main, bifurcating left main, and last patent coronary vessel) may the dose of clopidogrel be increased to 150 mg per day if <50% inhibition of platelet aggregation is demonstrated (8).

Although higher loading and maintenance doses of clopidogrel improve responsiveness, there is still a broad inter- and intravariability in the degree of antiplatelet effects achieved. In addition, results from the PREPARE POST-STENTING study show that ~50% of ischemic events occur in patients with post-treatment platelet reactivity within the 25th and 75th percentile distribution. These observations are suggestive that other thrombotic factors (i.e., thrombin generation), and not just platelet reactivity, may also play an important role in determining atherothrombotic events. This supports that inhibition of targets other than cyclooxygenase-1 and $P2Y_{12}$, in particular thrombin, need to be evaluated to reduce thrombotic risk (62).

Future Directions

The current therapeutic alternative for treatment of patients with poor clopidogrel response remains limited. Novel $P2Y_{12}$ receptor antagonists with more potent antiplatelet effects are currently under clinical investigation (77). These novel molecules are all characterized by more potent antiplatelet effects, reduced interindividual response variability, and therefore less likely to lead to resistance. Novel $P2Y_{12}$ receptor antagonists under clinical investigation include prasugrel, AZD6140, and cangrelor (Table 2).

Prasugrel (CS-747) is a member of the thienopyridine class of oral antiplatelet agents (78). Like ticlopidine and clopidogrel, prasugrel (a third-generation thienopyridine) is a pro-drug and needs to be transformed in the liver into an active metabolite. The active metabolite of prasugrel, like

Table 2 Novel $P2Y_{12}$ Receptor Antagonists Under Phase III Clinical Investigation

Drug	Type	Administration	Action	Dose	Mean Platelet Inhibition (Time Required)	Trial (Phase III)
Prasugrel (CS-747)	Thienopyridine (3rd generation)	Oral	Requires hepatic conversion to generate active metabolite Irreversible binding	60-mg loading dose, 10-mg maintenance dose	≈70% (<1 h)	TRITON
AZD-6140	Cyclopentyl-triazolo-pyrimidine	Oral	Direct inhibition Competitive binding	90 mg/twice daily	≈95% (2–4 h)	PLATO
Cangrelor (ARC-669931MX)	ATP analogue	Parenteral	Direct inhibition Competitive binding	4 μg/kg/min	≈95% (few minutes)	CHAMPION

ATP = adenosine triphosphate; CHAMPION = Cangrelor versus Standard Therapy to Achieve Optimal Management of Platelet Inhibition; CYP = cytochrome P450; PLATO = A Study of Platelet Inhibition and Patient Outcomes; TRITON = Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel.

the active metabolite of clopidogrel, leads to selective and irreversible blockade of the $P2Y_{12}$ receptor. However, compared with clopidogrel, prasugrel is more efficiently transformed into its active metabolite (79). A single oral administration of prasugrel produces a dose-related inhibition of platelet aggregation in rats approximately 10- and 100-fold more potent than that of clopidogrel and ticlopidine, respectively. Better degrees of platelet inhibition have also been confirmed in patients with coronary artery disease (80). The antiaggregatory effects of prasugrel are evident at 30 min and last until 72 h after dosing, indicating fast onset and long duration of action. The results of the JUMBO–TIMI (Joint Utilization of Medications to Block Platelets Optimally–Thrombolysis In Myocardial Infarction)-26 phase II trial showed that prasugrel has safety profiles (significant, non-coronary artery bypass grafting, bleeding through 30 days—primary end point of the study) comparable to standard-dose clopidogrel in patients (n = 900) undergoing PCI (81). Albeit not powered to assess clinical end points, encouraging clinical outcomes were observed in this trial. The ongoing TRITON (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel)–TIMI-38 phase III trial will compare prasugrel and clopidogrel in over 13,000 patients with ACS undergoing PCI with the primary end point of death, myocardial infarction, and stroke at 12 months. An additional study, the PRINCIPLE (Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation)–TIMI-44, is currently comparing the relative potency of prasugrel with a higher loading dose (600 mg) and maintenance dose (150 mg daily) of clopidogrel by assessing measures of platelet function, inflammation, and myocyte necrosis in patients undergoing elective PCI.

AZD6140 is the first oral reversible ADP receptor antagonist. It is a non-thienopyridine that belongs to a new chemical class called cyclopentyl-triazolo-pyrimidine. AZD6140 does not require hepatic metabolism for its activity and directly inhibits the $P2Y_{12}$ receptor (82). Platelet aggregation studies have shown that AZD6140 blocks platelet reactivity more consistently and completely than clopidogrel with a lower degree of interindividual response variability (83). The DISPERSE 2 (Safety, Tolerability and Preliminary Efficacy of AZD6140, the First Oral Reversible ADP Receptor Antagonist, Compared with Clopidogrel in Patients with Non–ST-Segment Elevation Acute Coronary Syndrome) was a phase II trial comparing AZD6140 with clopidogrel in patients (n = 990) with ACS that showed similar rates of bleeding in all groups (primary end point: total major/minor bleeding events at 4 weeks) and no significant difference in the composite end point of cardiovascular death, stroke, or recurrent ischemia (84). The ongoing PLATO (A Study of Platelet Inhibition and Patient Outcomes) phase III clinical trial is comparing AZD6140 and clopidogrel in ACS patients with the primary end point of death, myocardial infarction, and stroke at 12 months.

Cangrelor (also known as AR-C69931MX) is also a selective and competitive $P2Y_{12}$ antagonist, which is suitable for intravenous administration (85). Cangrelor is an ATP analogue, with more potent antiplatelet activity than clopidogrel (90% inhibition of platelet aggregation at 1 to 4 μg/kg/min intravenous) that leads to selective inhibition of ADP-induced aggregation in a dose-dependent manner. Importantly, there is a rapid reversal of its dose-dependent effects. Reports from phase II clinical trials show that cangrelor in addition to tissue-type plasminogen activator in patients with STEMI is associated with a greater degree of ST-segment recovery in a dose-dependent manner (85). Further, in patients undergoing PCI, cangrelor compares favorably with abciximab both from a safety and clinical standpoint. Two phase III trials with cangrelor (CHAMPION *PCI* and CHAMPION *PLATFORM*) are currently ongoing.

These novel and more potent $P2Y_{12}$ ADP receptor antagonists may have advantages over currently available antiplatelet agents, which are likely related to the increased degree of platelet inhibition (77). Increased platelet reactivity, in fact, is an important predictor of ischemic events. However, increased platelet inhibition not necessarily translates into better safety profiles as more potent antiplatelet agents may increase hemorrhagic risk. Results from phase III clinical trials will provide more definitive answers on the risk/benefits of these agents. Pre-clinical investigation of other ADP receptor antagonist such as INS-50589 (a dinucleotide intended for intravenous administration) and CT-50547 (a benzothiazolothiadiazine intended for oral administration) is also undergoing and will further nurture this evolving field of research with the goal of identifying the optimal treatment of patients with atherothrombotic disease undergoing PCI (77).

Conclusions

Poor responsiveness to antiplatelet agents, including clopidogrel, is an emerging clinical entity. Although there is increasing evidence that monitoring the effects of antiplatelet therapy may allow identification of patients at an increased risk of developing ischemic events, current clinical guidelines do not support routine screening for antiplatelet drug resistance. This is, in part, because the definition of the most appropriate platelet function assay has not been established and because of the lack of clinical trials showing the impact on clinical outcomes of treatment modification in patients resistant to antiplatelet agents. Currently, the most accurate platelet function assays are expensive, time-consuming, and not broadly available. Therefore, rapid and accurate diagnosis of responsiveness to antiplatelet agents also remains an issue. Widespread clinical application of assessment of antiplatelet drug response will require studies on large populations that define responsiveness in a standardized manner using assays with consistency and reproducibility, which correlate the measurements with clinical

outcomes, and that provide the strategies for modifying antiplatelet regimens to improve outcomes. Such tailored treatment regimens according to individual's need (through intensification or reduction of antithrombotic medication) will potentially reduce ischemic as well as bleeding risk in patients with hypo- and hyper-response, respectively, to standard treatment regimens. Defining the mechanisms leading to variability in responsiveness to antiplatelet agents, the best diagnostic tool for its evaluation, and the therapeutic measures for its treatment will hopefully set the future basis for routine measurements of platelet function and a new era of individualized antithombotic regimens (86).

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