Clopidogrel resistance?

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KEYWORDS
Clopidogrel; Responsiveness; Resistance; Platelet aggregation; P2Y12 receptor

Abstract Clopidogrel is an effective inhibitor of platelet activation and aggregation due to its selective and irreversible blockade of the P2Y12 receptor. Combination antiplatelet therapy with clopidogrel and aspirin is an important strategy for patients with acute coronary syndromes and those undergoing percutaneous interventions. Despite significant benefits demonstrated with combination antiplatelet treatment in large clinical trials, the occurrence of adverse ischemic events, including stent thrombosis, remains a serious clinical problem. Recent studies have demonstrated distinct response variability and nonresponsiveness to clopidogrel therapy based on ex vivo platelet function measurements. Small scale investigations have suggested that nonresponsiveness may be associated with a heightened risk for adverse clinical events. The above findings have stimulated a close examination of clopidogrel metabolism.

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Contents

Introduction ....................................................... 312
Mechanism of action................................................ 313
Clopidogrel resistance — definition ......................... 313
Laboratory evaluation of clopidogrel responsiveness........ 313
Clopidogrel responsiveness and time of treatment ........ 314
Clopidogrel responsiveness and effect of dose ............. 315
Relation of clopidogrel nonresponsiveness to adverse clinical events 315
Mechanism of clopidogrel resistance........................ 316
Management of clopidogrel resistance ....................... 318
Higher doses .................................................. 318
New P2Y12 receptor antagonists ............................. 318

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Introduction

Clopidogrel effectively inhibits ADP-induced platelet activation and aggregation by selectively and irreversibly blocking the P2Y12 receptor [1]. The CAPRIE study demonstrated the significant benefit of clopidogrel treatment in selected patients as compared to aspirin therapy alone [2]. Since clopidogrel and aspirin inhibit platelet aggregation through different pathways, combined antiplatelet therapy provides complementary and additive benefits compared to either agent alone. Clopidogrel treatment along with aspirin is considered the "gold standard" for attenuation of platelet activation and aggregation during acute coronary syndromes and in patients undergoing stenting [3–5]. In the United States, between 1998 and 2004, clopidogrel use has increased seven times whereas use of clopidogrel with aspirin has increased 16 times [6]. Despite significant benefits reported with combined antiplatelet treatment in large clinical trials, the occurrence of adverse ischemic events, including stent thrombosis, remains a serious clinical problem. Moreover, in the recent CHARISMA trial, the addition of clopidogrel to aspirin as a long term treatment strategy was found to be beneficial only in high risk patients with clinically evident atherothrombosis. In CHARISMA the effect of clopidogrel as a primary prevention strategy in addition to aspirin was associated with an increased risk of bleeding with no extra clinical benefits over aspirin therapy alone [7]. The recent ACTIVE W trial indicated that oral anticoagulation therapy was clearly superior to dual antiplatelet therapy with clopidogrel and aspirin for the prevention of vascular events in patients with atrial fibrillation at high risk of stroke [8]. The latter trials highlight the importance of understanding the pathophysiology of the disease state and targeting dual antiplatelet therapy to patients at high risk for arterial thrombotic events.

Figure 1  Mechanism of action of clopidogrel and laboratory evaluation of clopidogrel nonresponsiveness. Abbreviations; ADP — adenosine diphosphate, CYP3A4 — hepatic cytochrome 3A4, TxA2 — thromboxane A2, LC-MS/MS — liquid chromatography-mass spectrometry, LTA — light transmittance aggregometry, PRP — platelet rich plasma, TEG — thrombelastography, VASP-P — vasodilator-stimulated phosphoprotein-phosphorylated, PLA — platelet–leukocyte aggregation, PFA-100 — platelet function analyzer.
In addition, the previous clinical trials focussed on the reduction of clinical events following antiplatelet therapy without laboratory evaluation of platelet function. However, subsequent demonstrations of distinct response variability and nonresponsiveness to clopidogrel therapy, based on the laboratory evaluation of platelet response and the association of nonresponsiveness to adverse clinical events, demand a closer look at the effectiveness of clopidogrel treatment [9–12].

**Mechanism of action**

Clopidogrel inhibits ADP-induced platelet aggregation. Clopidogrel also inhibits collagen- and thrombin-induced aggregation; however, the inhibitory effect on collagen- and thrombin-induced aggregation can be overcome by increased concentrations of these agonists. These findings suggest that clopidogrel indirectly inhibits the effect of these agonists via the attenuation of ADP-mediated amplification of the platelet response [13].

Clopidogrel is rapidly absorbed from the intestine and extensively converted by hepatic cytochrome P450 isoenzymes (CYP3A4, CYP3A5, 2C19) to an active thiol metabolite [14,15]. This short lived active metabolite binds to the P2Y12 receptor via a disulfide bridge between the reactive thiol group and two cysteine residues (cys17 and cys270) present in the extracellular domains of the P2Y12 receptor. Thus, the binding of ADP to the P2Y12 receptor is permanently inhibited [1](Fig. 1). Clopidogrel has also been reported to attenuate platelet–leukocyte aggregate formation, and the levels of CRP, p-selectin and CD 40L; and the rate of thrombin formation [16–20].

**Clopidogrel resistance — definition**

No single receptor signaling pathway mediating platelet activation is responsible for all thrombotic complications. Therefore, a single treatment strategy directed against a specific receptor cannot overcome all thrombotic complications. With this in mind, it is our opinion that the optimal definition of resistance or nonresponsiveness to an antiplatelet agent is the failure of the antiplatelet agent to inhibit the target of its action. The identification of resistance would therefore utilize a laboratory technique that detects residual activity of the target. Therefore, clopidogrel resistance is best demonstrated by evidence of residual post-treatment P2Y_{12} activity by measuring ADP-induced platelet aggregation before and after treatment. Since thrombosis involves multiple signaling pathways, treatment failure is not synonymous with drug resistance (BOX-1).

**Laboratory evaluation of clopidogrel responsiveness**

A standardized laboratory method that simulates the in vivo platelet response to antiplatelet therapy is still lacking. Since clopidogrel specifically inhibits one of two ADP receptors, ex-vivo measurement of ADP-induced maximum platelet aggregation by light transmittance aggregometry (LTA) has been the most commonly used laboratory method to evaluate clopidogrel responsiveness and is considered the gold standard [9]. Recently, it was suggested that since antiplatelet drugs (especially clopidogrel), induce platelet disaggregation, the response to clopidogrel would be better demonstrated by measuring late platelet aggregation at 6 min after stimulation with ADP rather than maximum aggregation [20]. However, unpublished data from our laboratory, based on the evaluation of both maximum and final aggregation from 100 consecutive patients undergoing stenting and treated with clopidogrel, indicated that both measurements were equivalent in determining the prevalence of clopidogrel nonresponsiveness. Flow cytometric measurements of the expression of activated GP IIb/IIIa receptor and p-selectin expression after ADP stimulation can also identify clopidogrel nonresponsiveness and correlated with measurements of maximum aggregation stimulated by ADP [9,21]. In addition, measurements of ADP-induced platelet-fibrin clot strength by whole blood thrombelastography and the VerifyNow P2Y_{12} receptor assay using ADP as the agonist can also be used to measure clopidogrel responsiveness as point-of-care assays [22,23]. The PFA-100 method using collagen-ADP based cartridges and whole blood aggregometry are associated with inconsistent estimates of platelet reactivity to ADP. The phosphorylation state of vasodilator-stimulated phosphoprotein is a specific intracellular marker of residual P2Y_{12} receptor.

<table>
<thead>
<tr>
<th>Antplatelet Drug Non-responsiveness/ Resistance</th>
<th>=</th>
<th>Failure to Inhibit Target</th>
</tr>
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<tbody>
<tr>
<td>Antplatelet Drug Non-responsiveness/ Resistance</td>
<td>≠</td>
<td>Clinical Failure</td>
</tr>
</tbody>
</table>

No single pathway mediates all thrombotic events - Multiple pathways of platelet activation

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Box 1

<table>
<thead>
<tr>
<th>Clopidogrel Non-responsiveness</th>
<th>= Absolute Change in Aggregation Inhibition ≤ 10% (Absolute Change in Aggregation = Max. baseline aggregation - Max. post-drug aggregation)</th>
</tr>
</thead>
</table>

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313

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reactivity in patients treated with clopidogrel and can be measured by flow cytometry. This technique is perhaps the most specific indicator of residual P2Y12 activity in patients treated with a P2Y12 inhibitor. However, the methodology is labor intensive and requires permeation of the platelet membrane and use of monoclonal antibodies specific for phosphorylated vasodilator-stimulated phosphoprotein [21,24] (Fig. 2).

Clopidogrel responsiveness and time of treatment

Similar to other drugs, response variability and nonresponsiveness to clopidogrel have been demonstrated in patients following coronary stenting [9,25]. In an early investigation from our center, 96 patients undergoing elective stenting were treated with a 300 mg clopidogrel loading dose in the catheterization laboratory followed by a 75 mg maintenance dose. ADP-induced platelet aggregation and activation dependent platelet surface marker expression (p-selectin and activated GPIIb/IIIa) were assessed at baseline and serially for 30 days following stenting. Response variability to clopidogrel was demonstrated as measured by all markers and a certain percentage of patients were found to have no demonstrable antiplatelet effect [9]. In the latter patients, the difference between pre- and post-treatment ADP-induced platelet aggregation was ≤10%. We defined these patients as clopidogrel “resistant” or “nonresponsive” to clopidogrel therapy. A subgroup of resistant patients exhibited platelet aggregation that was greater after stent implantation than at baseline despite clopidogrel therapy. These patients were defined as having heightened platelet reactivity to ADP.

In the latter study, 53–63% of patients were resistant to clopidogrel treatment at 2 h post-

![Figure 2](image-url) Relationship between frequency of patients and absolute change in aggregation (Δ Aggregation [%]) in response to 5 uM ADP at 2 h (A), 24 h (B), days (C), and 30 days (D) after stenting. Δ Aggregation (%) is defined as baseline aggregation (%) minus post-treatment aggregation (%). Resistance, as defined therein, is Δ Aggregation ≤10%. Resistance is present in those patients subtended by double-headed arrow. Curves represent normal distribution of data. (Adapted from, Gurbel et al. Circulation 2003;107:2908–13).

<table>
<thead>
<tr>
<th>Investigators</th>
<th>n</th>
<th>Patients</th>
<th>Clopidogrel dose (mg, load/qd)</th>
<th>Definition of clopidogrel resistance</th>
<th>Time</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gurbel et al [9]</td>
<td>92 PCI</td>
<td>300/75</td>
<td>5 and 20 uM ADP-induced aggregation &lt;10% absolute change</td>
<td>24 h</td>
<td>31–35%</td>
<td></td>
</tr>
<tr>
<td>Jaremo et al [27]</td>
<td>18 PCI</td>
<td>300/75</td>
<td>ADP-induced fibrinogen binding &lt;40% of baseline</td>
<td>24 h</td>
<td>28%</td>
<td></td>
</tr>
<tr>
<td>Muller et al [28]</td>
<td>119 PCI</td>
<td>600/75</td>
<td>5 and 50 uM ADP-induced aggregation &lt;10% relative change</td>
<td>4 h</td>
<td>5–11%</td>
<td></td>
</tr>
<tr>
<td>Mobely et al [29]</td>
<td>50 PCI</td>
<td>300/75</td>
<td>1 uM ADP-induced aggregation, TEG and lchor PW: &lt;10% absolute inhibition</td>
<td>Pre and post</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Lepantalo et al [30]</td>
<td>50 PCI</td>
<td>300/75</td>
<td>2 or 5 uM AD-induced aggregation and PFA-100 10% inhibition and 170s</td>
<td>2.5 h</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>Angiolillo et al [31]</td>
<td>48 PCI</td>
<td>300/75</td>
<td>6 uM ADP-induced aggregation &lt;40% inhibition</td>
<td>10 min, 4 and 24 h</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Matetzky et al [32]</td>
<td>60 STEMI</td>
<td>300/75</td>
<td>5 uM ADP-induced aggregation and CPA &lt;10% inhibition</td>
<td>Daily for 5 days</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td>Dziewierz A et al [33]</td>
<td>31 CAD</td>
<td>300</td>
<td>20 uM ADP-induced aggregation &lt;10% absolute change</td>
<td>24 h</td>
<td>23%</td>
<td></td>
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<tr>
<td>Lev EI et al [34]</td>
<td>150 PCI</td>
<td>300</td>
<td>5 uM ADP-induced aggregation &lt;10% absolute change</td>
<td>20–24 h</td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td>Angiolillo et al [35]</td>
<td>52 Diabetics and non-diabetics</td>
<td>300</td>
<td>5 and 20 uM ADP-induced aggregation &lt;10% absolute inhibition</td>
<td>24 h</td>
<td>38% diabetics 8% non-diabetics</td>
<td></td>
</tr>
<tr>
<td>Gurbel et al [25]</td>
<td>190 PCI</td>
<td>300 or 600/75</td>
<td>5 and 20 uM ADP-induced aggregation &lt;10% absolute inhibition</td>
<td>24 h</td>
<td>28–32% with 300 mg 8% with 600 mg</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations—PCI=percutaneous coronary interventions; ADP=adenosine diphosphate; CAD=Coronary artery disease TEG=thrombelastography; PW=Plateletworks; PFA-100=Platelet function analyzer-100; CPA=Cone and platelet analyzer.
stenting; \( \sim 30\% \) were resistant at day 1 and day 5 post-stenting; and 13–21% were resistant at day 30 post-stenting [9] (Fig. 2). Therefore, clopidogrel “resistance” in this study appeared to be time dependent. Based on the results we hypothesized that the occurrence of clopidogrel resistance might be related to the inadequacy of a 300 mg loading dose to provide sufficient active metabolite generation to arrest platelet reactivity in selected patients, and that these resistant patients may be at particular risk for thrombotic complications including periprocedural infarction and stent thrombosis [9,22,26].

Since this initial description, multiple investigators have confirmed the phenomenon of clopidogrel resistance [27–35]. The prevalence of clopidogrel nonresponsiveness has been reported at 5–44%. This wide variation in prevalence is primarily due to dosing and is less related to various definitions, laboratory methods, and the time at which blood samples were evaluated for responsiveness [27–35] (Table 1). As demonstrated in Table 1, the data are markedly concordant. The higher resistance estimates are present following the 300 mg loading dose and the lower estimates occur after the 600 mg loading dose [25,28]. Diabetic patients who were on long term dual antiplatelet therapy had a higher number of clopidogrel nonresponders compared to nondiabetic patients in a recent study [35].

Clopidogrel responsiveness and effect of dose

Subsequent investigations have unequivocally demonstrated that clopidogrel nonresponsiveness is dependent on dose. In the largest pharmacodynamic study comparing 300 mg and 600 mg clopidogrel loading doses, treatment with a 600 mg loading dose during elective PCI reduced clopidogrel nonresponsiveness to 8% compared to 28–32% after a 300 mg loading dose (Fig. 3). Moreover, the study demonstrated a narrower response profile following treatment with 600 mg compared to 300 mg clopidogrel [25]. Müller et al also observed a time and dose dependent effect of clopidogrel in patients undergoing stenting [28]. A similar increased responsiveness was also observed in the ISAR-CHOICE study, where a ceiling effect of platelet inhibition was observed with a 600 mg clopidogrel loading dose whereas a nonsignificant increase in platelet inhibition was observed with a 900 mg loading dose [36].

Relation of clopidogrel nonresponsiveness to adverse clinical events

Limited data are available to link clopidogrel nonresponsiveness to the occurrence of thrombotic events. Matetzky et al studied clopidogrel responsiveness in patients undergoing stenting for acute ST-elevation myocardial infarction and found that patients who exhibited the highest quartile of ADP-induced aggregation had a 40% probability for a recurrent cardiovascular event within 6 months [32]. In the PREPARE POST-STENTING (Platelet REactivity in Patients And Recurrent Events POST-STENTING) Study, patients suffering a recurrent ischemic event within 6 months of elective stenting had high post-stent platelet reactivity to ADP compared to patients without ischemic events despite dual antiplatelet therapy [22]. In the CLEAR PLATELETS (Clopidogrel Loading with Eptifibatide to Arrest PLATELET reactivity) and CLEAR PLATELETS Ib Studies, a 600 mg clopidogrel loading dose used to treat patients undergoing elective stenting was associated with superior early platelet inhibition compared to a 300 mg loading dose and this inhibition was accompanied by a decrease in release of myocardial necrosis and inflammation markers [37,38]. Cuisset et al demonstrated that patients with high post-treatment platelet reactivity despite dual antiplatelet therapy [22]. In the CLEAR PLATELETS (Clopidogrel Loading with Eptifibatide to Arrest PLATELET reactivity) and CLEAR PLATELETS Ib Studies, a 600 mg clopidogrel loading dose used to treat patients undergoing elective stenting was associated with superior early platelet inhibition compared to a 300 mg loading dose and this inhibition was accompanied by a decrease in release of myocardial necrosis and inflammation markers [37,38]. Cuisset et al demonstrated that patients with high post-treatment platelet reactivity had an increased risk of cardiovascular events. More importantly, these patients were resistant to both clopidogrel and aspirin treatment [39]. Similarly Lev et al demonstrated that occurrence of creatinine kinase-myocardial band after stenting was more frequent in patients exhibiting aspirin and clopidogrel resistance [34]. Finally, significantly higher recurrent ischemic events within 6 months of the procedure were observed in patients who were on chronic clopidogrel therapy undergoing elective coronary stenting and had higher pre-
procedure ADP-induced platelet aggregation [40] (Table 2). All these findings strongly suggest that a high platelet reactivity despite currently recommended antiplatelet therapy is a risk factor for ischemia in patients undergoing PCI.

Based on the analysis of flow cytometric measurements of intracellular VASP phosphorylation levels, a specific intracellular marker of clopidogrel-induced P2Y12 receptor inhibition, nonresponsiveness to clopidogrel treatment has been suggested as a risk factor for the occurrence of stent thrombosis [21,41]. In the recent CREST (Clopidogrel effect on platelet Reactivity in patients with Stent Thrombosis) Study, elevated levels of ADP-induced platelet aggregation, ADP-stimulated expression of active GPIIb/IIIa expression and the P2Y12 reactivity ratio measured by VASP phosphorylation were observed in patients with stent thrombosis compared to patients without stent thrombosis, indicating inadequate inhibition of P2Y12 receptor [21]. Other investigators have reported that high ex vivo shear-induced platelet aggregation despite dual antiplatelet therapy may be a risk factor for stent thrombosis [42] (Table 2).

### Clinical relevance of clopidogrel nonresponsiveness

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Results</th>
<th>Clinical relevance</th>
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</thead>
<tbody>
<tr>
<td><strong>Post-stent ischemic events and periprocedural infarction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Matetzky et al. [32]</td>
<td>60</td>
<td>↑ ADP-induced platelet aggregation (4th quartile)</td>
<td>Recurrent cardiac events</td>
</tr>
<tr>
<td>2. Gurbel et al. [22] (PREPARE Post-Stenting Study)</td>
<td>192</td>
<td>↑ Periprocedural platelet aggregation</td>
<td>Post-PCI ischemic events (6 months)</td>
</tr>
<tr>
<td>3. Gurbel et al. [37,38] (CLEAR PLATELETS and CLEAR PLATELETS lb)</td>
<td>120</td>
<td>↑ Periprocedural platelet aggregation</td>
<td>Myonecrosis and inflammation marker release</td>
</tr>
<tr>
<td>4. Bliden et al. [40]</td>
<td>100</td>
<td>↑ Periprocedural platelet aggregation in patients on chronic clopidogrel</td>
<td>Post-PCI ischemic events (6 months)</td>
</tr>
<tr>
<td>5. Cuisset et al. [39]</td>
<td>106</td>
<td>↑ Platelet aggregation</td>
<td>Recurrent events</td>
</tr>
<tr>
<td>6. Lev et al. [34]</td>
<td>120</td>
<td>↑ Clopidogrel/ aspirin resistant patients</td>
<td>Post-PCI myonecrosis</td>
</tr>
<tr>
<td><strong>Stent thrombosis</strong></td>
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<tr>
<td>7. Barragen et al. [41]</td>
<td>36</td>
<td>↑ P2Y12 reactivity ratio (VASP-levels)</td>
<td>Stent thrombosis</td>
</tr>
<tr>
<td>8. Gurbel et al. [21] (CREST Study)</td>
<td>120</td>
<td>↑ P2Y12 reactivity ratio</td>
<td>Stent thrombosis</td>
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<td></td>
<td></td>
<td>↑ platelet aggregation</td>
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<td>↑ stimulated GPIIb/IIIa expression</td>
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<tr>
<td>9. Ajzenberg et al. [42]</td>
<td>49</td>
<td>↑ Shear-induced platelet aggregation</td>
<td>Stent thrombosis</td>
</tr>
</tbody>
</table>

ADP = adenosine diphosphate; CLEAR PLATELETS Study = clopidogrel loading with epifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading With Epifibatide to Arrest the Reactivity of Platelets study; CREST Study = clopidogrel effect on platelet reactivity in patients with stent thrombosis; GP = glycoprotein; PCI = percutaneous coronary intervention; PREPARE POST-Stenting Study = platelet reactivity in patients and recurrent events post-stenting study; VASP = vasodilator-stimulated phosphoprotein.

### Mechanism of clopidogrel resistance

The mechanisms responsible for clopidogrel response variability and resistance are incompletely defined. Differences in intestinal absorption, hepatic conversion to the active metabolite through cytochrome 3A4 (CYP3A4) activity, and platelet receptor polymorphisms have been suggested [14,43–46].

Only up to 30–50% inhibition of ex vivo ADP-induced platelet aggregation was demonstrated following a repeated daily dose of 75 mg in normal volunteers or loading doses of 300 or 600 mg in patients undergoing PCI [13,37,38]. This level of inhibition indicated an incomplete P2Y12 receptor blockade and suboptimal inhibition of ADP-induced platelet aggregation. The repeated demonstrations that a high loading dose of 600 mg clopidogrel is associated with increased inhibition of ex vivo ADP-induced platelet aggregation in patients undergoing PCI and a decreased prevalence of nonresponders support insufficient active metabolite generation as a major factor in clopidogrel resistance [25,28,36,43,47].

Recent studies involving the measurement of hepatic cytochrome (CYP) P450 activity suggest that individual variations in the activity of this enzyme play a major role [14,48–50]. In a landmark investigation, Lau et al demonstrated that pharmacologic stimulation of CYP3A4 activity by rifampin enhances the inhibitory effect of clopidogrel, whereas agents that compete with clopidogrel for CYP 3A4 activity (e.g. erythromycin) attenuate the antiplatelet effect of clopidogrel [14,48]. Additional information supporting the pivotal role of CYP P450 in generating the active metabolite of clopidogrel was demonstrated in a small study involving human volunteers where the effects of the CYP3A4 inhibitor, ketoconazole, on the pharmacokinetics
and the ex vivo platelet inhibitory effects of prasugrel and clopidogrel were investigated. Prasugrel is also a thienopyridine that requires conversion to an active metabolite by a hepatic cytochrome. In this study, ketoconazole co-administration did not have any effect on prasugrel active metabolite generation or prasugrel-induced platelet inhibition, whereas clopidogrel-induced platelet inhibition was reduced following a loading dose as well as after a maintenance dose [49]. The latter effect on clopidogrel-induced platelet inhibition was accompanied by lesser active metabolite generation. Similarly, in another study, prasugrel treatment was associated with superior active metabolite generation and platelet inhibition together with a lower incidence of nonresponsiveness compared to clopidogrel treatment [50].

In recent studies, the influence of CYP3A5 and CYP2C19 isoenzymes on clopidogrel metabolic activation and responsiveness has been demonstrated [51,52]. Suh J et al demonstrated higher clopidogrel responsiveness among subjects with the CYP3A5 expressor genotype than subjects with the non-expressor genotype. Moreover, worse outcomes were seen in patients undergoing stent implantation with the non-expressor genotype following treatment with clopidogrel than in patients with the CYP3A5 expressor genotype [51]. Similarly, Hulto J et al and Brandt et al independently demonstrated the influence of the CYP2C19 genotype on clopidogrel responsiveness in healthy volunteers [52]. Finally, Angiolillo DJ et al demonstrated that the IVS10+12G>A polymorphism of the CYP3A4 gene may be an important contributor to clopidogrel response variability [53] (Fig. 4).

Suboptimal platelet response to clopidogrel may be due to an increased number of platelet P2Y12 receptors, or polymorphism of platelet receptors. Genetic polymorphisms of platelet GPIb/IIa, GPIa/IIa, or P2Y12 receptors have been reported to affect platelet function and may influence clopidogrel response variability [54–56]. Recently, it was reported that an increased percentage of patients with peripheral arterial disease have the P2Y12 receptor H2 haplotype [55]. However, in another study, the relation of this haplotype to clopidogrel responsiveness could not be demonstrated [56]. Since the relation of genetic polymorphisms to clopidogrel responsiveness is inconclusive, further studies are required to establish a correlation between receptor polymorphisms and clopidogrel nonresponsiveness.

It has been shown that patients with diabetes exhibit platelet activation and increased reactivity to agonists. The heightened platelet reactivity may be related to the increased prevalence of nonresponders and occurrence of ischemic events reported in patients with diabetes [57,58]. It has also been reported that patients with a high body mass index (BMI) exhibited a suboptimal platelet response with the standard 300 mg loading dose [59].

All of the above data strongly support the importance phenomenon of insufficient metabolite generation secondary to limitations in the intestinal absorption, drug–drug interaction at CYP 3A4 or genetic polymorphisms of CYP isoenzymes as the...
Management of clopidogrel resistance

Higher doses

In recent clinical studies of patients undergoing stenting, a 600 mg clopidogrel loading dose was associated with a higher level of platelet inhibition, lower mean post-treatment reactivity to ADP, and a lower incidence of nonresponsiveness when compared to a 300 mg dose [25,28,37,60]. Moreover, a 600 mg clopidogrel loading dose was associated with a narrower response profile [25]. Kastrati et al found that patients achieved additional platelet inhibition when a 75 mg/day clopidogrel maintenance dose was followed by an additional 600 mg loading dose [61]. In the CLEAR PLATELETS Study a 600 mg loading dose was associated with a superior pharmacodynamic antiplatelet profile compared to a 300 mg clopidogrel loading dose [37,38]. In the recent ISAR-CHOICE (Intracoronary Stenting and Antithrombotic Regimen: Choice Between 3 High Oral doses for Immediate Clopidogrel Effect) Study, there was a ceiling effect in unchanged clopidogrel and clopidogrel metabolite levels and platelet inhibition with the 600 mg loading dose, and no significant additional effect was seen with the 900 mg loading dose. Based on the pharmacokinetic profile of the free drug and metabolites the investigators concluded that intestinal absorption was the major factor explaining response variability [62].

Thus, higher loading doses may be considered for selected patients exhibiting high platelet reactivity to ADP. However, the superiority of a high dose regimen in reducing ischemic events and the associated risk profile compared to a standard dose has yet to be established in large scale clinical trials. Despite these limitations, the current ACC/AHA guidelines for PCI provide a Class IIa recommendation that "a regimen of greater than 300 mg is reasonable to achieve higher levels of antiplatelet activity more rapidly". Finally, the ACC/AHA Guidelines provide a Class IIb recommendation that "in patients in whom subacute thrombosis may be catastrophic or lethal... platelet aggregation studies may be considered and the dose of clopidogrel increased to 150 mg per day if less than 50% inhibition of platelet aggregation is demonstrated" [63]. The latter guidelines however, do not specify the methodology that should be used to assess inhibition. Moreover, there are very limited clinical data to support the cutpoint of 50% inhibition [21,37]. In the CLEAR PLATELETS Study we observed periprocedural myocardial infarction only in those patients with 5 µM ADP-induced aggregation >50% [37]. In the CREST Study the cutpoint for stent thrombosis was 20 µM ADP-induced aggregation >40% [21].

New P2Y12 receptor antagonists

New P2Y12 receptor antagonists are currently undergoing investigation. AZD 6140 (Astra-Zeneca) and cangrelor (Medicines Company) are reversible, direct and potent inhibitors of the P2Y12 receptor [64,65]. AZD 6140 is an oral inhibitor whereas cangrelor is administered parenterally. Both of these agents exhibit more consistent and greater platelet inhibition compared to clopidogrel. The short onset and offset of action make these agents appealing adjunctive antiplatelet agents during PCI when maximum and rapid platelet inhibition of ADP-induced aggregation is desired [64,65]. Prasugrel is an irreversible inhibitor of P2Y12 and, similar to clopidogrel, is a prodrug that requires metabolic activation. In the JUMBO-TIMI-26 trial, prasugrel treatment was associated with a similar primary endpoint of significant bleeding compared to standard clopidogrel regimen (1.7 vs. 1.2) and the bleeding events were the same for all doses of prasugrel [66]. The pharmacodynamic profile of prasugrel is superior to clopidogrel and is associated with lesser incidences of nonresponsiveness [67]. All three of the above agents will undergo study in phase 3 clinical trials. Prasugrel is currently being compared to clopidogrel in an ACS trial in patients undergoing PCI.

Conclusion

Clopidogrel use has increased over the last few years following its effectiveness together with aspirin in significantly reducing adverse events in large-scale clinical trials. At the same time, based on the laboratory evaluation of platelet response, wide response variability and nonresponsiveness in selected patients are also present. In the recent small studies, heightened platelet reactivity or clopidogrel nonresponsiveness in patients who were on clopidogrel treatment was associated with adverse thrombotic events including stent thrombosis. The primary reason has been attributed to the suboptimal generation of active metabolite secondary to potential limitation in intestinal absorption, drug–drug interaction at CYP3A4 and genetic polymorphism of hepatic cytochrome P450 isoenzymes. Use of higher loading or maintenance doses of clopidogrel or new and more potent P2Y12 receptor blockers is a potential alternative strategy. In addition, treatment with combined antiplatelet...
therapies may be confined to the pharmacologic management of patients at high risk for arterial thrombotic events but not as a primary prevention strategy or as an alternative to antiplatelets.

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