**P2Y₁₂ Antagonism**

**Promises and Challenges**

Alan D. Michelson

**Abstract**—The P2Y₁₂ antagonist clopidogrel has a well-established role as an antithrombotic agent in the settings of percutaneous coronary intervention and acute coronary syndromes. However, several challenges remain, including the relatively slow onset of action of clopidogrel and the phenomenon of clopidogrel response variability or “resistance”. Novel P2Y₁₂ antagonists, including prasugrel, AZD6140, and cangrelor, have a faster onset of action, as well as more potent, and less variable, inhibition of platelet function ex vivo. Whether this promise will be translated into clinical benefit for patients will be determined by the results of ongoing phase 3 clinical trials. (*Arterioscler Thromb Vasc Biol. 2008;28:000-000.)*

**Key Words:** platelets ■ clopidogrel ■ prasugrel ■ AZD6140 ■ cangrelor

Platelets normally circulate in a resting form. In response to a vascular injury, eg, rupture of an atherosclerotic plaque in a coronary artery, platelets adhere to the damaged vessel wall, become activated, and aggregate with one another, resulting in a platelet-dependent thrombus. The goal of antiplatelet therapy is to prevent or treat this platelet-dependent thrombus. This article reviews a clinically important category of antiplatelet drugs: the P2Y₁₂ antagonists (Table 1).

Adenosine diphosphate (ADP), an important platelet agonist in vivo, has 2 types of receptors in the platelet plasma membrane: P2Y₁ and P2Y₁₂.¹ P2Y₁ is a 7-transmembrane receptor linked to a G protein (Figure 1A). The end result of ADP signaling through its P2Y₁ receptor is calcium mobilization, platelet shape change, and rapidly reversible platelet aggregation. P2Y₁₂ is also a 7-transmembrane domain receptor, but it is linked to a G inhibitory protein (Figure 1A). The end result of ADP signaling through its P2Y₁₂ receptor is amplification of stable platelet aggregation and secretion.

**Currently-Approved P2Y₁₂ Antagonists**

The 2 currently FDA-approved P2Y₁₂ antagonists, ticlopidine and clopidogrel, are thienopyridines (Table 1) that are metabolized through cytochrome P450 in the liver. The thienopyridine metabolites (Figure 2A), not the parent ticlopidine or clopidogrel molecules, irreversibly antagonize the P2Y₁₂ receptor (Figure 1A, Table 1). Ticlopidine, the first FDA-approved P2Y₁₂ antagonist, is given orally twice a day. However, in the United States and most other countries, ticlopidine has been largely replaced in clinical practice by clopidogrel, given orally in a more convenient daily dose, because of the better side-effect profile of clopidogrel—for example, less neutropenia and a lower incidence of the rare but dangerous thrombotic thrombocytopenic purpura.²

Clopidogrel is well established as an antiplatelet therapy.² Large multicenter randomized controlled trials have demonstrated its benefit. The CURE (Clopidogrel in Unstable angina to prevent Recurrent Events) trial was performed in 12,562 patients with acute coronary syndrome, unstable angina, or non-ST elevation myocardial infarction.³ These patients were randomized to either a clopidogrel loading dose of 300 mg or placebo followed by clopidogrel 75 mg daily plus aspirin 75 to 325 mg daily or placebo plus aspirin 75 to 325 mg daily. Patients were followed for 12 months, with a primary end point of myocardial infarction, stroke, and cardiovascular death, and there was a relative risk reduction (RRR) of 20% in the clopidogrel-treated group (P<0.001). The PCI-CURE (Percutaneous Coronary Intervention CURE) study was a continuation of the CURE study.³ Those 2658 patients who went on to PCI received open-label thienopyridine and 30 days post-PCI were randomized to either clopidogrel plus aspirin or placebo plus aspirin and then followed for 12 months. Based on a composite end point of cardiovascular death or myocardial infarction from randomization to the end of follow-up in PCI-CURE, patients treated with clopidogrel had a 31% RRR compared with patients treated with placebo (P=0.002). The CREDO (Clopidogrel for the Reduction of Events During Observation) trial confirmed the beneficial effect of clopidogrel in post-PCI patients.⁵ Subsequently, the COMMIT (Clopidogrel and Metoprolol in Myocardial Infarction Trial) and CLARITY-TIMI 28 (CLOpidogrel as Adjunctive ReperfusIon TherapY-Thrombolysis In Myocardial Infarction 28) trials demonstrated the benefit of...
clopidogrel and aspirin in patients with ST-elevation myocardial infarction.\textsuperscript{6,7} However, in patients with stable cardiovascular disease or asymptomatic patients with multiple cardiovascular risk factors, the 15 603-patient CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance) trial reported that the combination of clopidogrel plus aspirin was not significantly more effective than aspirin alone in reducing the rate of myocardial infarction, stroke, or death from cardiovascular causes.\textsuperscript{8} Furthermore, the risk of moderate-to-severe bleeding was increased.\textsuperscript{8} In a retrospective analysis of the CHARISMA trial, dual antiplatelet therapy with clopidogrel and aspirin in the primary prevention subgroup of patients was

Table 1. P2Y\textsubscript{12} Antagonists

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Direct or Indirect</th>
<th>Reversible</th>
<th>Route</th>
<th>Frequency</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ticlopidine</td>
<td>Thienopyridine</td>
<td>Indirect</td>
<td>No</td>
<td>PO</td>
<td>Twice daily</td>
<td>Approved</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>Thienopyridine</td>
<td>Indirect</td>
<td>No</td>
<td>PO</td>
<td>Daily</td>
<td>Approved</td>
</tr>
<tr>
<td>Prasugrel</td>
<td>Thienopyridine</td>
<td>Indirect</td>
<td>No</td>
<td>PO</td>
<td>Daily</td>
<td>3</td>
</tr>
<tr>
<td>AZD6140</td>
<td>ATP analog</td>
<td>Direct</td>
<td>Yes</td>
<td>PO</td>
<td>Twice daily</td>
<td>3</td>
</tr>
<tr>
<td>Cangrelor</td>
<td>ATP analog</td>
<td>Direct</td>
<td>Yes</td>
<td>IV</td>
<td>...</td>
<td>3</td>
</tr>
<tr>
<td>PRT060128</td>
<td>...</td>
<td>Direct</td>
<td>Yes</td>
<td>PO, IV</td>
<td>...</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 1. A, Mechanism of action of P2Y\textsubscript{12} antagonists. The 2 currently FDA-approved P2Y\textsubscript{12} antagonists, ticlopidine and clopidogrel, are thienopyridines which are metabolized through cytochrome P450 in the liver. The thienopyridine metabolites, not the parent ticlopidine or clopidogrel molecules, irreversibly antagonize the P2Y\textsubscript{12} receptor. B, Vasodilator-stimulated phosphoprotein (VASP) assay for the measurement of P2Y\textsubscript{12} antagonism. PGE\textsubscript{1} binds to its IP receptor on the platelet surface and signals through a G stimulatory (Gs) protein and adenylyl cyclase (AC) to convert ATP to cAMP, and then through protein kinase A (PKA) to convert VASP to phosphorylated VASP (VASP-P). ADP binds to its P2Y\textsubscript{12} receptor on the platelet surface and signals through a G inhibitory (Gi) protein to inhibit PGE\textsubscript{1}-induced signaling through AC. P2Y\textsubscript{12} antagonists, eg, the active metabolite of clopidogrel, inhibit this ADP-induced effect. Therefore, in the presence of both PGE\textsubscript{1} and ADP, VASP-P is directly proportional to the degree of P2Y\textsubscript{12} antagonism. VASP-P is measured by whole blood flow cytometry, using permeabilization and a monoclonal antibody specific for the phosphorylated form of VASP. Modified with permission from Cattaneo.\textsuperscript{1}

Figure 2. A, Thienopyridines and their active metabolites. B, ATP and its analogs. Reproduced with permission from Cattaneo.\textsuperscript{2}
associated with an increase in cardiovascular death. The cause of this apparent harm has not been elucidated.

**Clopidogrel Response Variability**

P2Y$_{12}$ antagonist therapy can be monitored in patients by a number of methods (Table 2). Arguably the most specific method currently available for the monitoring of P2Y$_{12}$ antagonist therapy in patients is the flow cytometric measurement of the phosphorylation of vasodilator stimulated phosphoprotein (VASP, BioCytex). Under the conditions of this assay, which uses a combination of ADP and prostaglandin (PG) E$_{1}$ (Figure 1B), the phosphorylation of VASP (identified by a monoclonal antibody specific for the phosphorylated form of VASP) is directly proportional to the degree of inhibition of the P2Y$_{12}$ receptor. The major advantage of the VASP assay is its direct dependence on the target of clopidogrel, P2Y$_{12}$ (Figure 1B).

Alternatively, P2Y$_{12}$ antagonist therapy can be monitored by ex vivo stimulation of the platelets with ADP and then reading out one of a number of end points including platelet aggregation (Table 2). Comparison of the VASP assay with ADP-induced turbidometric platelet aggregation demonstrated that the measured level of thienopyridine-induced inhibition is higher in the VASP assay, presumably because platelet aggregation can still occur via ADP stimulation of P2Y$_{12}$ in the presence of a thienopyridine. The VerifyNow P2Y12 Assay (Accumetrics) has a number of advantages, including (1) like the VASP phosphorylation assay, it uses a combination of ADP and PGE$_{1}$, and (2) it is a point-of-care device that can produce a rapid readout at the patient’s bedside.

Monitoring of clopidogrel by each of the assays listed in Table 2 reveals interpatient response variability. Furthermore, there is evidence that a poor response in such in vitro assays (ie, clopidogrel hyporesponsiveness or “resistance”) predict a poor clinical response to clopidogrel—as evidenced by major adverse clinical events (MACE). Thus, clopidogrel hyporesponsiveness, defined by ADP-induced turbidometric platelet aggregation, VASP phosphorylation, or the TEG PlateletMapping System, has been reported to be associated with post-PCI MACE. However, it is important to emphasize that the number of adverse clinical events was low in all these studies.

**How Should a Patient With Clopidogrel Hyporesponsiveness or “Resistance” be Managed?**

Noncompliance is certainly an important consideration to be addressed. Interference by other drugs metabolized via cytochrome P450 may be a consideration, but the evidence for a clinically-important effect is not strong. Because no published studies address the clinical effectiveness of altering

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**Table 2. Platelet Function Tests for Measuring Response to Clopidogrel**

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2Y$_{12}$-specific</td>
<td>VASP phosphorylation (flow cytometry)</td>
</tr>
<tr>
<td>ADP-stimulated</td>
<td>Platelet aggregometry (turbidometric)</td>
</tr>
<tr>
<td></td>
<td>Platelet aggregometry (impedance)</td>
</tr>
<tr>
<td></td>
<td>VerifyNow P2Y12 Assay</td>
</tr>
<tr>
<td></td>
<td>Plateletworks</td>
</tr>
<tr>
<td></td>
<td>TEG PlateletMapping System</td>
</tr>
<tr>
<td></td>
<td>Impact cone and plate(let) analyzer</td>
</tr>
<tr>
<td></td>
<td>Platelet surface activated GPIIb-IIIa, platelet surface P-selectin, leukocyte-platelet aggregates (flow cytometry)</td>
</tr>
</tbody>
</table>

**Table 3. Possible Mechanisms of Clopidogrel Response Variability or “Resistance”**

<table>
<thead>
<tr>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability</td>
</tr>
<tr>
<td>Noncompliance</td>
</tr>
<tr>
<td>Underdosing</td>
</tr>
<tr>
<td>Poor absorption</td>
</tr>
<tr>
<td>Interference by other drugs</td>
</tr>
<tr>
<td>Platelet Function</td>
</tr>
<tr>
<td>Accelerated platelet turnover, with introduction into the bloodstream of newly formed, drug-unaffected platelets</td>
</tr>
<tr>
<td>Increased platelet sensitivity to ADP and collagen</td>
</tr>
<tr>
<td>Single Nucleotide Polymorphisms</td>
</tr>
<tr>
<td>P2Y$<em>{12}$, P2Y$</em>{1}$, cytochrome P450</td>
</tr>
<tr>
<td>Other Factors</td>
</tr>
<tr>
<td>Smoking, hypercholesterolemia, etc</td>
</tr>
</tbody>
</table>

Rather Than Clopidogrel Response Variability or Resistance, Is It:

- Treatment failure (because arterial thrombosis is multifactorial)?
- Platelet response variability?

Modified with permission from Michelson, Circulation. 2004;110:e489.
therapy based on a laboratory finding of clopidogrel resistance, the correct treatment, if any, of clopidogrel hyporesponsiveness or “resistance” remains unknown. Nevertheless, the current American College of Cardiology/American Heart Association PCI guidelines have a Class Ib recommendation based on level C evidence that, in patients in whom subacute stent thrombosis may be catastrophic or lethal, platelet aggregation studies may be considered and the maintenance dose of clopidogrel increased from 75 mg to 150 mg per day if less than 50% inhibition of platelet aggregation is demonstrated. Although there are as yet no published clinical outcomes studies to support this approach, a 150 mg daily maintenance dose of clopidogrel has recently been shown to provide more effective platelet inhibition (as determined by ADP-induced turbidometric platelet aggregation, the VerifyNow P2Y12 Assay, and VASP phosphorylation) than the current standard maintenance dose of 75 mg daily.

However, even at the higher maintenance dose of 150 mg daily there is still a large variability in the degree of platelet inhibition, and the possible increased hemorrhagic risks of this approach have not been studied. In the PCI setting, a loading dose of 600 mg of clopidogrel, rather than the previous standard loading dose of 300 mg, has been widely adopted based on small studies showing more rapid and profound inhibition of ADP-induced turbidometric platelet aggregation, reduced myonecrosis markers, and reduced MACE at 30 days. An increase in the clopidogrel loading dose from 600 mg to 900 mg may result in an additional significant increase in inhibition of platelet function. However, even at these higher clopidogrel loading doses of 600 mg and 900 mg, there is still large variability in the degree of platelet inhibition. The ongoing CURRENT/OASIS 7 (Clopidogrel optimal loading dose Usage to Reduce Recurrent Events/Optimal Antiplatelet Strategy for InterventionS 7) trial (www.clinicaltrials.gov/ct/show/NCT00335452) may help clarify the optimal loading and maintenance doses of clopidogrel.

**Novel P2Y<sub>12</sub> Antagonists**

Continuing interrelated challenges with regard to clopidogrel treatment include (1) the still significant incidence of stent thrombosis in patients treated with clopidogrel and aspirin, (2) the phenomenon of clopidogrel hyporesponsiveness or “resistance”, and (3) the relatively slow onset of action of clopidogrel, with little antithrombotic benefit from a 300-mg loading dose given less than 12 hours before PCI. A number of novel P2Y<sub>12</sub> antagonists (Table 1) are therefore under investigation to determine whether they can result in better or more rapid antithrombotic effects than clopidogrel, without an unacceptable increase in hemorrhagic (or other) side effects.

Prasugrel (Eli Lilly & Co./Daiichi Sankyo) is an investigational orally-administered thienopyridine prodrug that, like clopidogrel, is metabolized via cytochrome P450 in the liver (Figure 2A). The active metabolite of prasugrel irreversibly inhibits the platelet P2Y<sub>12</sub> receptor to a similar extent to the active metabolite of clopidogrel. However, there is much more efficient in vivo generation of the active metabolite of prasugrel than of the active metabolite of clopidogrel. As a result, a prasugrel 60 mg loading dose results in a much more rapid, potent, and consistent inhibition of platelet function than the standard clopidogrel loading dose of 300 mg and the more recently adopted clopidogrel loading dose of 600 mg. Furthermore, a maintenance dose of prasugrel 10 mg daily results in a more potent and consistent inhibition of platelet function than the standard clopidogrel maintenance dose of 75 mg daily.

The 13 608-patient phase 3 trial, TRITON-TIMI 38 (TRial to Assess Improvement in Therapeutic Outcomes by optimizing platelet inhibition with prasugrel), was recently completed. This trial demonstrated that in patients with acute coronary syndromes with scheduled PCI, prasugrel (60 mg loading dose and a 10 mg daily maintenance dose), as compared with approved doses of clopidogrel (300 mg loading dose and a 75 mg daily maintenance dose), was associated with significantly reduced rates of ischemic events, including stent thrombosis, but with an increased risk of major bleeding, including fatal bleeding. The primary efficacy end point occurred in 12.1% of patients receiving clopidogrel and 9.9% of patients receiving prasugrel (hazard ratio for prasugrel versus clopidogrel, 0.81; 95% confidence interval [CI], 0.73 to 0.90; P<0.001). There were also significant reductions in the prasugrel group in the rates of myocardial infarction (9.7% for clopidogrel versus 7.4% for prasugrel; P<0.001), urgent target-vessel revascularization (3.7% versus 2.5%; P<0.001), and stent thrombosis (2.4% versus 1.1%; P<0.001). Major bleeding was observed in 2.4% of patients receiving prasugrel and in 1.8% of patients receiving clopidogrel (hazard ratio, 1.32; 95% CI, 1.03 to 1.68; P=0.03). Also greater in the prasugrel group was the rate of life-threatening bleeding (1.4% versus 0.9%; P=0.01), including nonfatal bleeding (1.1% versus 0.9%; hazard ratio, 1.25; P=0.23) and fatal bleeding (0.4% versus 0.1%; P=0.002). A posthoc subgroup exploratory analysis of the data identified 3 subgroups of interest that had less clinical efficacy and greater absolute levels of bleeding than the overall cohort, resulting in less net clinical benefit or in clinical harm. These subgroups were: patients with a history of stroke or transient ischemic attack, age ≥75 years, and body weight <60 kg.

The PRINCIPLE-TIMI 44 trial demonstrated that, among patients undergoing cardiac catheterization with planned PCI, a 60 mg prasugrel loading dose resulted in greater platelet inhibition than the now widely used higher clopidogrel loading dose of 600 mg. Maintenance therapy with prasugrel 10 mg daily resulted in a greater antiplatelet effect than the high clopidogrel maintenance dose of 150 mg daily. This trial was not powered for clinical outcomes.

AZD6140 (AstraZeneca) is another investigational P2Y<sub>12</sub> antagonist (Table 1). To increase oral bioavailability, the structure of AZD6140 was modified from AR-C109318XX (Figure 2B). Unlike ticlopidine, clopidogrel, and prasugrel, AZD6140 is (1) not a thienopyridine but an ATP analog (Figure 2B), (2) a direct P2Y<sub>12</sub> antagonist (ie, no metabolism of a prodrug is required), and (3) a reversible P2Y<sub>12</sub> antagonist. Like prasugrel, AZD6140: (1) results in a more rapid
onset of action and greater degree of platelet inhibition than clopidogrel, (2) maintenance therapy results in more potent inhibition of platelet function than the standard clopidogrel maintenance dose of 75 mg daily, (3) showed no significant increase in bleeding compared with clopidogrel in phase 2 studies.48–50 In these phase 2 studies, dyspnea was greater, in an apparently dose-dependent manner, in patients on AZD6140 compared with patients on clopidogrel. AZD6140 is given orally twice a day and is currently in a phase 3 trial: PLATO (PLATElet inhibition and patient Outcomes).

Cangrelor (The Medicines Company) is an investigational, direct-acting, reversible P2Y12 antagonist (Table 1, Figure 2B). Unlike the above-described orally-administered P2Y12 antagonists (ticlopidine, clopidogrel, prasugrel, and AZD6140), cangrelor is administered intravenously—which, together with the rapid reversal of its effects after the end of the infusion, may be potentially advantageous in the PCI setting. Like prasugrel and AZD6140, cangrelor results in a more rapid onset of action and greater degree of platelet inhibition than clopidogrel, and showed no significant increase in bleeding compared with clopidogrel in phase 2 studies.51,52 Cangrelor is currently in phase 3 trials: CHAMPION-PCI and CHAMPION-PLATFORM.

PRT060128 (Portola) is an investigational, direct-acting, reversible P2Y12 antagonist with a novel structure.53 PRT060128, which can potentially be administered orally or intravenously, has completed phase 1 clinical studies.

Conclusions

The P2Y12 antagonist clopidogrel has a well-established role as an antithrombotic agent in the settings of PCI and acute coronary syndromes. However, several challenges remain, including the relatively slow onset of action of clopidogrel and the phenomenon of clopidogrel response variability or “resistance”. Novel P2Y12 antagonists, including prasugrel, AZD6140, and cangrelor have a faster onset of action, as well as more potent, and less variable, inhibition of platelet function ex vivo. Whether this promise will be translated into clinical benefit for patients will be determined by the results of phase 3 clinical trials.

Disclosures

None.

References


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