Understanding Resistance to Thienopyridine Antiplatelet Therapy

Targeting the antiplatelet effect with platelet function testing is a novel therapeutic approach that requires investigation.

BY MATTHEW J. PRICE, MD

Dual-antiplatelet therapy with aspirin and a thienopyridine is the cornerstone of therapy for patients with atherosclerosis. Aspirin therapy is protective for patients at risk for vascular occlusion and reduces recurrent events in patients with established coronary artery disease (CAD).[1] Compared to aspirin monotherapy, the combination of aspirin and a thienopyridine improves outcomes in patients undergoing PCI[2,3] who present with non-ST elevation acute coronary syndrome (ACS)[4] or ST-elevation myocardial infarction (MI),[5] and in patients with a history of MI, ischemic stroke, or symptomatic peripheral arterial disease.[6] Furthermore, premature discontinuation of thienopyridine therapy after drug-eluting stent (DES) implantation is associated with a markedly increased hazard for stent thrombosis.[7] However, there is substantial variability among individuals in the pharmacodynamic effects of ticlopidine and clopidogrel. Prospective, observational studies have demonstrated that a poor inhibitory response and/or high residual platelet reactivity on clopidogrel therapy is associated with adverse outcomes after PCI. Recognition of this phenomenon, when appropriately defined and tested, may help risk stratify patients, and in the future, may lead to personalized antiplatelet therapy for patients with cardiovascular disease.

LABORATORY ASSESSMENT OF THE EFFECT OF ANTIPLATELET THERAPY

The molecular target of the thienopyridines is the platelet $P2Y_{12}$ receptor. This receptor mediates the completion and amplification of the platelet aggregatory response when activated by its agonist, adenosine 5'-diphosphate (ADP).[8] The thienopyridines are prodrugs that require conversion to an active metabolite through a multistep process mediated by the hepatic cytochrome P450 (CYP) system. This active metabolite irreversibly binds and inhibits the $P2Y_{12}$ receptor for the life of the platelet.

The antiplatelet effect of thienopyridines can be measured ex vivo by several methods (Table 1). The magnitude of $P2Y_{12}$ receptor activation, through its effect on adenyl cyclase (and also in the concentration of cyclic adenosine monophosphate), is proportional to the phosphorylation status of vasodilator-stimulated phosphoprotein (VASP), which can be measured by flow cytometry. Light transmittance aggregometry (LTA) can provide an ex vivo measurement of the antiaggregatory effect of clopidogrel by measuring the transmission of light through platelet-rich plasma after exposure to ADP (using platelet-poor plasma as a reference).[9] Newer assays, such as the point-of-care VerifyNow P2Y12 assay

(Accumetrics, San Diego, CA) and the near-bedside thrombelastography with PlateletMapping system (Haemoscope Corporation, Niles, IL) require less specialized equipment and technical training and have been found to correlate well with traditional methods such as LTA.[10,11]

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The antiaggregatory effect of an antiplatelet agent can be described either by the absolute or relative change in aggregation before and after antiplatelet exposure (ie, "the inhibition of platelet aggregation [IPA]") or by the "residual platelet reactivity" during thienopyridine therapy (also referred to as "on-treatment reactivity"), which only requires a single measurement after antiplatelet initiation. Although both measurement approaches can be used to describe an antiplatelet effect, they are not interchangeable; they may identify different sets of patients with potentially different risks of subsequent events.[12] The effect of clopidogrel varies remarkably among individuals, whether measured by IPA, residual platelet reactivity, or the VASP platelet reactivity index; this variability cannot be overcome even by large loading doses (Figure 1).[13-15] The mechanism underlying this variability is multifactorial but appears to be, in large part, due to loss-of-effect polymorphisms of the CYP system that reduce efficiency of the generation of clopidogrel's active metabolite (in particular, CYP2C19*2),[16] polymorphisms of the drug-efflux transporter gene ABCB1,[17] the presence of diabetes mellitus,[18] and possibly, concomitant medications or other factors that may interfere with the hepatic biotransformation of clopidogrel.[19-22]

DEFINING THIENOPYRIDINE RESISTANCE

The terms *nonresponsiveness*, *high residual platelet reactivity*, or *high on-treatment reactivity* have been used to categorize patients with a poor clopidogrel response as measured by platelet aggregation studies or VASP phosphorylation analysis (Table 2). Many studies have defined poor clopidogrel effect a priori using an operator-defined or population-based threshold (eg, <10% IPA, ADP-induced aggregation >70%, VASP index >50%, lowest quartile of IPA, highest quartile of on-treatment platelet reactivity). When defined in such a manner, a poor clopidogrel effect has been significantly associated with short- and longer-term ischemic events in patients undergoing PCI for stable CAD and ACS[10,23-30] and with cardiovascular events in chronically treated patients with CAD and diabetes mellitus.[31]

A more powerful way to define a clinically relevant threshold for platelet function after clopidogrel exposure is to determine an optimal cutoff that maximizes the sum of the sensitivity and specificity for subsequent clinical events using receiver-operator characteristic curve analysis.[32] Studies using receiver-operator characteristic curve analysis have shown that the VASP platelet reactivity index, LTA, thrombelastography platelet function mapping, and the VerifyNow P2Y12 assay can identify clopidogrel-treated patients at risk for subsequent events after PCI.[10,31,34,35] Three studies using the VerifyNow P2Y12 assay involving a total of more than 1,000 patients have demonstrated a consistent optimal diagnostic cutoff of residual platelet reactivity >235–240 $P2Y_{12}$ reaction units; this threshold identified approximately one-third of patients and was prognostic for periprocedural, 6-month, and 1-year events after PCI.[33,35,36] Residual reactivity below this cutoff had excellent negative predictive value[36] consistent with the findings of other studies using LTA or VASP phosphorylation analysis.[34,37] The prognostic value and optimal cutoff for the VerifyNow P2Y12 assay will be further examined by the Assessment of Dual-AntiPlatelet Therapy with Drug-Eluting Stents registry, which will prospectively evaluate the relationship

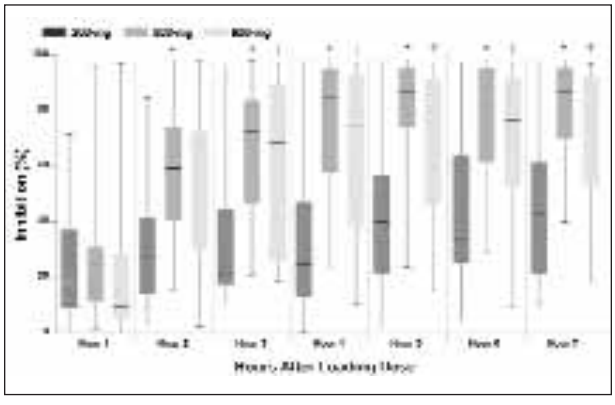

Figure 1. Change in platelet reactivity (% inhibition) over time after a clopidogrel 300-, 600-, and 900-mg loading dose. Subjects receiving 600 or 900 mg have significantly greater inhibition than those receiving 300 mg at 2 hours after administration, although there is no difference in inhibition between 600 and 900 mg at any time point. Note the substantial variability in inhibition at all time points regardless of the magnitude of the loading dose. (P*<.05 between 300 and 600 mg; †*P*<.05 between 300 and 900 mg.) (Adapted from Price MJ, et al. Am J Cardiol. 2006;98:681-684.[14])**

TABLE 1. COMMONLY USED ASSESSMENTS OF THE ANTIPLATELET EFFECT OF THIENOPYRIDINE THERAPY

Terminology	Method of Assessment	What Is Being Measured
ADP-induced aggregation (%)	LTA	Absolute level of platelet reactivity in response to ADP (% is in reference to control [platelet poor plasma])
Inhibition of platelet aggregation (%)	LTA	Change in ADP-induced aggregation before and after thienopyridine exposure
$P2Y_{12}$ reaction units	VerifyNow P2Y12 Assay	Absolute level of platelet reactivity in response to ADP
VASP platelet reactivity index	Flow cytometry	Magnitude of $P2Y_{12}$-receptor activity

LTA, light transmittance aggregometry; ADP, adenosine 5'-diphosphate; VASP, vasodilator-stimulated phosphoprotein.

TABLE 2. TERMS USED TO DESCRIBE THE ANTIPLATELET EFFECT OF THIENOPYRIDINE THERAPY

Term	Sampling Requirements	Common Definition
Nonresponsiveness	Blood sample and measurement before and after thienopyridine exposure	Little or no change in ADP-induced platelet aggregation before and after thienopyridine exposure
High residual platelet reactivity, high on-treatment reactivity	Single blood sample and measurement while on thienopyridine therapy	Platelet reactivity while on thienopyridine therapy (eg, aggregation %, $P2Y_{12}$ reaction units) above a particular threshold

between the results of the VerifyNow P2Y12 assay and ischemic events, including stent thrombosis, in more than 11,000 patients undergoing PCI with DES.

TREATING THIENOPYRIDINE RESISTANCE

There are several potential options in treating patients on clopidogrel therapy with high residual platelet reactivity and/or poor IPA. First, medication noncompliance, if present, must be addressed. Second, assuming that the patient has been compliant, the clopidogrel maintenance dose may be increased. Repeated loading doses of 600 mg daily for up to 4 days can generally overcome nonresponsiveness according to VASP phosphorylation analysis.[30] An additional clopidogrel 600-mg loading dose followed by 150 mg per day has been shown to significantly reduce platelet aggregation in patients with acute MI who have high ADP-induced platelet aggregation on a standard maintenance therapy of 75 mg per day.[38] In a randomized study of diabetic patients who were poor responders, a 150-mg maintenance dose enhanced the antiplatelet effect of clopidogrel, although this effect was nonuniform, and some patients had persistently elevated residual reactivity.[39] The third option is to add additional antiplatelet agents. Cilostazol reduces ADP-induced platelet aggregation by LTA and $P2Y_{12}$-receptor activity by VASP phosphorylation analysis, although the incremental effect of cilostazol on $P2Y_{12}$ inhibition in clopidogrel nonresponders has not been well studied. The use of adjunctive glycoprotein IIb/IIIa inhibitors in clopidogrel nonresponders in the acute setting is also being examined.[40] As a fourth option, one could potentially switch $P2Y_{12}$ inhibitors; nonresponsiveness to both clopidogrel and ticlopidine is uncommon,[41] and newer $P2Y_{12}$-inhibitors, such as prasugrel and AZD6140, provide greater and more consistent inhibition than clopidogrel.[42]

It is important to realize that the safety and efficacy of altering antiplatelet management in patients with a poor clopidogrel effect has not been examined, and therefore, there are currently no clinical data to support such a treatment strategy. The Gauging Responsiveness with A VerifyNow Assay-Impact on Thrombosis And Safety trial is an international, randomized, placebo-controlled clinical trial that will enroll approximately 2,800 patients with stable angina/ischemia or non-ST elevation acute coronary syndrome undergoing PCI with DES. Patients with high residual platelet reactivity on clopidogrel therapy, according to the VerifyNow Assay 12 to 24 hours post-PCI, will be randomized to standard maintenance clopidogrel therapy (75 mg daily) or high-dose clopidogrel therapy (additional loading dose followed by 150 mg daily) for 6 months. A random sample of patients without high residual reactivity will be followed and treated with standard clopidogrel therapy. The primary endpoint of the trial is the time to first occurrence of cardiovascular death, nonfatal MI, or definite/probable stent thrombosis. This study will help

determine whether individualized antiplatelet therapy based on routine platelet function testing will safely improve outcomes after PCI.

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MOVING FROM THE IDENTIFICATION OF RESISTANCE TO THE TARGETING OF THERAPY

The concept of thienopyridine resistance arose from the observation that there is wide variation in the inhibitory effect of ticlopidine and clopidogrel among individuals and that events after PCI appear to cluster in patients with the highest residual reactivity. Newer $P2Y_{12}$ antagonists, such as prasugrel, provide greater and more consistent inhibition and reduce ischemic events compared with clopidogrel but at the cost of increased bleeding.[43] However, the cardiac event rates in most clopidogrel-treated patients (ie, those without high residual reactivity) are quite low. The absolute risk reduction achieved by treating patients who have an adequate clopidogrel response with a more powerful $P2Y_{12}$ antagonist may be relatively small, while potentially exposing them to a bleeding hazard. Therefore, the use of platelet function testing to target a particular level of platelet reactivity is an attractive hypothesis that must be tested in clinical trials.

CONCLUSION

There is wide interindividual variability in the inhibitory effect of clopidogrel and ticlopidine. Cardiac events after PCI, including recurrent ACS, are more frequent in patients with high on-treatment reactivity. Although potential therapeutic options for such patients include increasing the maintenance dose, adding additional antiplatelet agents, or changing to newer, more powerful and consistent $P2Y_{12}$ antagonists, the safety and clinical efficacy of such a personalized strategy must be tested in randomized, clinical trials. The observation that the majority of patients on clopidogrel therapy appear to be at low risk argues against a one-size-fits-all strategy with newer $P2Y_{12}$ inhibitors. Targeting of the antiplatelet effect with platelet function testing represents a novel therapeutic approach that merits investigation. ■

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