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Aspirin, P2Y₁₂ blockers, cilostazol, PAR-1 blockers and emerging antiplatelet therapies: can biomarkers guide clinical development and practice?

“...functional testing ... is increasingly recognized as a useful biomarker to describe drug characteristics and guide both early and later clinical trial programs. In this unique issue of Biomarkers in Medicine, global experts in the field carefully describe the strengths, and the limitations, of functional testing for several established and emerging antiplatelet agents.”

Ian BA Menown

Craigavon Cardiac Centre, Southern Trust, Northern Ireland, UK
ian.menown@southerntrust.hscni.net

Over the past two decades, large randomized clinical trials have helped define the current standard of care for patients with acute coronary syndromes, including those undergoing urgent percutaneous coronary intervention [1,2]. For the majority of patients, this will include dual antiplatelet therapy; typically aspirin and a platelet adenosine diphosphate P2Y₁₂ receptor blocker. Some patients may also receive a third antiplatelet therapy, most commonly intravenous glycoprotein IIb/IIIa blockade, although clinical experience is growing with the use of other oral antiplatelets on top of conventional dual antiplatelet therapy.

While a significant number of ischemic events may still occur despite guideline-based practice, clinical trials with conventional antiplatelet therapies suggest a ceiling has been reached, beyond which further increases in efficacy are associated with unacceptable increases in bleeding.

Functional testing of new & established antiplatelet agents

One approach has been the development of novel antiplatelets as alternatives, or add-ons, to clopidogrel. Advances in understanding of platelet activation, amplification and aggregation has enabled identification of multiple potential therapeutic targets (FIGURE 1). New therapies include third-generation P2Y₁₂ blockers (e.g., prasugrel, ticagrelor, cangrelor and elinogrel), direct thromboxane A₂ antagonists (terutroban and picotamide), inhibition of collagen and ristocetin-mediated platelet function (DZ-697b), the phosphodiesterase III inhibitor (cilostazol) and protease-activated receptor-1 blockers (e.g., vorapaxar and atopaxar). Novel oral antithrombins (including dabigatran) and anti-Xa agents (including rivaroxaban and

apixaban), which reduce platelet activation via a reduction in thrombin activation, are also being tested in patients with acute coronary syndrome.

Ex vivo and *in vivo* functional testing, tailored to drug mechanism of action, is increasingly recognized as a useful biomarker to describe drug characteristics and guide both early and later clinical trial programs. In this unique issue of *Biomarkers in Medicine*, global experts in the field carefully describe the strengths, and the limitations, of functional testing for several established and emerging antiplatelet agents.

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Measuring antiplatelet activity of aspirin

Foo and Oldroyd [3] and Schrör *et al.* [4] describe multiple methods for evaluating the antiplatelet function of aspirin (an irreversible inhibitor of cyclooxygenase-1-dependent thromboxane A₂ formation), including inhibition of arachidonic acid-mediated platelet aggregation, assessing the surface expression of platelet activation markers, such as P-selectin, integrins (glycoprotein IIb/IIIa) or CD40 ligand, and measurement of serum thromboxane B₂ or its urinary metabolite, 11-dehydro-thromboxane B₂.

Assessing response to P2Y₁₂ blockers

Price *et al.* give a detailed description of the principal methodologies of laboratory-based and point-of-care tests to assess the antiplatelet effect of P2Y₁₂ blockers by ADP-induced platelet aggregation (which acts mainly, but not exclusively, via P2Y₁₂) or more specific measures of P2Y₁₂

Figure 1. Targets for platelet inhibition showing sites of action of established and emerging therapies.

5HT: 5-hydroxytryptamine; GP: Glycoprotein; LMWH: Low-molecular-weight heparin; PAR: Protease-activated receptor; TP: Thromboxane A₂/prostaglandin H₂; UFH: Unfractionated heparin.

Adapted with permission from [10].

receptor reactivity using a vasodilator-stimulated phosphoprotein phosphorylation technique [5]. Application of such methods are key for understanding the clinical antiplatelet effects of clopidogrel in conventional or higher doses (Foo and Oldroyd [3]), prasugrel (Jakubowski [6]) and ticagrelor (Shand *et al.* [7]).

Lippi *et al.* describe methods for assessing the antiplatelet effect of glycoprotein IIb/IIIa inhibitors, including ADP-induced platelet aggregation, closure time (measurement of platelet hemostatic capacity) and platelet activation markers [8].

Individualization of therapy?

A second emerging approach is greater individualization of therapy. While dose adjustment according to weight, age or renal function (as with some parenteral therapies) is not yet commonplace for oral agents, recent clinical trials have attempted to define patient subgroups in whom dose modification may be of net clinical benefit [9].

Foo and Oldroyd [3] and Schrör *et al.* [4] highlight the considerable inter- and intra-patient variability in aspirin response and the clinical significance of aspirin hyporesponsiveness. However, the absence of standardized methodologies for assessment of aspirin antiplatelet activity, the lack of linear correlation between inhibition of thromboxane A₂ formation and platelet function, and the unclear association with clinical outcome means that such biomarkers are not yet ready to guide individualized aspirin therapy and research opportunity remains.

Several of this issue's articles [3,5–7] highlight the considerable inter- and intra-patient variability in the degree of platelet inhibition following clopidogrel with high on-treatment residual ADP-induced platelet aggregation being predictive of further ischemic events. Clopidogrel hyporesponsiveness is probably multifactorial, including genetic, cellular and clinical factors. Clopidogrel is susceptible to polymorphism of the *ABCB1* gene (which

affects absorption) and cytochrome P450 2C19 and 3A4 genes (which affects levels of active metabolite). Approximately 30% of the population carries a 'loss-of-function' 2C19 allele, which is probably of greatest importance in a post-percutaneous coronary intervention setting, particularly if homozygous. The clinical significance of drug interaction with high-dose lipophilic statins or certain proton-pump inhibitors remains unclear, but patient comorbidity, including acute coronary syndromes, diabetes or elevated BMI, has been associated with reduced clopidogrel responsiveness.

A practical approach

Genetic testing has the advantage of being a fixed and clear result, but as even the commonest polymorphism (2C19) accounts for only a minority of the variability in clopidogrel response, it is best reserved to complement clinical/functional risk assessment rather than as a standalone tool. In the setting of dual

therapy with aspirin and clopidogrel, high residual on-treatment platelet reactivity may be associated with poorer outcome. As doubling the dose of clopidogrel only provides a modest pharmacodynamic effect [5], switching to a more potent P2Y₁₂ blocker or adding a third agent, such as cilostazol or a protease activated receptor-1 blocker, may be appropriate providing the patient is not at high bleeding risk. Studies are underway testing the clinical value of such strategies.

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