

## Identifying clopidogrel resistance during chronic therapy: The case for a biochemical approach

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The thienopyridine derivative clopidogrel is now routinely utilised to reduce risk of coronary stent thrombosis, and the drug has also demonstrated efficacy in reducing cardiac events in patients with stable symptomatic vascular disease and acute coronary syndromes even in the absence of stent implantation. In general, clopidogrel is used in combination with low-dose aspirin, and has proved to be well-tolerated in the vast majority of patients. However, in recent years there has been increasing concern that the occurrence of thrombotic events in patients receiving clopidogrel, both early in the course of treatment and during long-term therapy, may reflect failure of the drug to adequately inhibit platelet aggregation. In particular, there is increasing evidence of a nexus between risk of stent thrombosis and “clopidogrel resistance” (1).

Central to the widening debate regarding optimal utilisation of clopidogrel is the evidence of heterogeneity of anti-aggregatory response, both acutely and during chronic therapy. Many factors contribute to this heterogeneity of response, which is more marked in patients with ischaemic heart disease than in normal subjects. From a pharmacokinetic point of view, clopidogrel is a pro-drug which may be subject to heterogeneous rates of activation: kinetics may be affected markedly by drug interactions and pharmacogenetic variability. However, there are also concerns regarding inter-individual variability of the pharmacological effects of clopidogrel based on the biochemical cascade of events modified by its blockade of the platelet P2Y<sub>12</sub> receptors.

Stimulation of the P2Y<sub>12</sub> receptor by ADP leads to suppression of platelet adenylyate cyclase activity and a consequent fall in cyclic AMP generation: this in turn limits phosphorylation of vasodilator-stimulated phosphoprotein (VASP), causing activation of glycoprotein IIb/IIIa. Clopidogrel therefore functions to “rescue” adenylyate cyclase, restoring responsiveness to agonists such as prostacyclin. Not surprisingly, lack of responsiveness to clopidogrel at the level of ADP-induced platelet aggregation is directly correlated with impaired effects of clopidogrel in stimulating VASP phosphorylation responses to prostacyclin and other agonists. The bases for this “failure” of biochemical signal cascade modulation extend beyond the pharmacokinetics of

clopidogrel, and probably include interactions with the various stimuli for adenylyate cyclase and soluble guanylate cyclase activation. For example, platelets from patients with angina pectoris exhibit relative resistance from a physiological and biochemical standpoint to the effects of both nitric oxide and prostacyclin (2), both of which stimulate VASP phosphorylation.

Thus, alterations in VASP-phosphorylation state in the presence of clopidogrel do not merely represent the biochemical effects of clopidogrel, nor do they correspond fully with the determinants of ADP-induced platelet aggregation. Indeed, an additional consideration limiting the utility both of VASP-based and aggregation-based methodology *in vitro* is that both approaches capture only in part the determinants of platelet aggregation and thrombus formation *in vivo*. Specifically, it is uncertain, despite the established predictive capacity of such methodology *in vitro*, to what extent it correlates with platelet aggregation induced predominantly by stimuli other than ADP.

One possible approach to the clinical problem of clopidogrel resistance is to increase dosage in all patients. This is increasingly popular with the loading dose (600 mg vs. 300 mg), but in the vast majority of patients the maintenance dose has been maintained at 75 mg. Indeed, it appears that even doubling of the maintenance dose would not restore appropriate responsiveness in all patients (3).

An alternative – and increasingly evidence-based – approach involves individualisation of dosage of physiological or biochemical response. Physiological response determination requires measurement of platelet aggregation response to ADP, and is most suitable for acute titration of clopidogrel effect, for example during loading in cases of coronary emergency and stent implantation for acute myocardial ischaemia. The biochemical correlates of this process, which are addressed in the currently published study by Schäfer et al. (4), may be monitored by evaluating clopidogrel effects on agonist-induced VASP phosphorylation. Studies of this type usually quantitate a platelet reactivity index (PRI), which varies inversely with prostaglandin E<sub>1</sub> induced VASP phosphorylation: PRI values of >50% suggest inadequate response to clopidogrel.

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In the currently published study (4), Schäfer et al. focused on the problem of assessing clopidogrel response during chronic therapy. A total of 100 patients with stable coronary disease receiving 75 mg/day clopidogrel were evaluated: a cohort of 33 similar patients treated with aspirin alone were utilised as a comparator group. The main finding of the study was that non-responsiveness to clopidogrel was significantly more frequently detected by the VASP-based assay than by measuring residual ADP-induced aggregation in platelet-rich plasma, although the results of these two evaluations were moderately well correlated with each other: overall criteria for inadequate response to clopidogrel were present in approximately 70% of patients.

The study also extends information related to clinical correlates of platelet resistance to clopidogrel: low levels of plasma high-density lipoprotein (HDL) and a history of hyperlipidaemia were independently associated with clopidogrel resistance, extending previous observations suggesting that diabetes mellitus may also carry similar associations.

The lack of a close correlation between residual aggregation-based and VASP-based indices of clopidogrel effect is both con-

sistent with previous observations and with the fact that ADP-induced aggregation is not purely modulated via VASP phosphorylation. The considerably greater prevalence of clopidogrel resistance in patients than in normal subjects also probably reflects disordered homeostasis of platelet function in such patients.

However, the results of the study by Schäfer et al. (4) highlight the emerging issue of the optimal method for monitoring individual patient responses to clopidogrel (or to other emerging anti-aggregatory agents). A recently published study has demonstrated that in patients with baseline resistance to clopidogrel, upward titration of dosage guided by serial PRI estimation is associated with reduced risk of major adverse events after coronary stenting (5). The results of the study by Schäfer et al. provide a further impetus towards routine monitoring of individual response to clopidogrel during chronic therapy. However, the relative utility of biochemical versus aggregometry-based monitoring, as against selective or routine global increases in clopidogrel maintenance dose, remains to be evaluated in appropriately sized comparative studies.

## References

1. Wenaweser P, Dorffler-Melly J, Imboden K, et al. Stent thrombosis is associated with an impaired response to antiplatelet therapy. *J Am Coll Cardiol* 2005; 45: 1748–1752.
2. Chirkov YY, Chirkova LP, Sage RE, et al. Impaired responsiveness of platelets from patients with stable angina pectoris to antiaggregating and cyclic AMP-elevating effects of prostaglandin E1. *J Cardiovasc Pharmacol* 1995; 25: 961–966.
3. Fontana P, Senouf D, Mach F. Biologic effect of increased maintenance dose of clopidogrel in cardiovascular outpatients and influence of the cytochrome P450 2C19\*2 allele on clopidogrel responsiveness. *Thromb Res* 2008; 121: 463–468.
4. Schafer A, Weinberger S, Flierl U, et al. ADP-induced platelet aggregation frequently fails to detect impaired clopidogrel-responsiveness in patients with coronary artery disease compared to a P2Y<sub>12</sub>-specific assay. *Thromb Haemost* 2008; 100: 618–625.
5. Bonello L, Camoin-Jau L, Arques-S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. *J Am Coll Cardiol* 2008; 51: 1404–1411.