

Clopidogrel Resistance

a report by

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Dual antiplatelet therapy with aspirin and thienopyridines (clopidogrel or ticlopidine) has become increasingly important for the management of patients with ischaemic vascular disease. The combination of aspirin and clopidogrel has become the standard for the prevention of sub-acute stent thrombosis and protection against ischaemic events in a variety of clinical scenarios including acute coronary syndromes (ACS) and ST-elevation myocardial infarctions (STEMIs).¹⁻⁶ However, clinical experience with clopidogrel and aspirin suggests that some patients do not exhibit the expected response and recurrent events on aggressive therapy are common. In fact, in the recently reported Superior Yield of the New Strategy of Enoxaparin, Revascularization and Glycoprotein IIb/IIIa inhibitors (SYNERGY) trial, more than 10% of patients presenting with high-risk ACS treated with 'triple antiplatelet therapy' (aspirin, clopidogrel and glycoprotein IIb/IIIa inhibition) and with intensive anti-thrombin therapy suffered a recurrent MI at 30 days.⁷ The concept that some patients might be resistant to the effects of aspirin and/or clopidogrel has been advanced to explain the high recurrent event rate noted in trials such as SYNERGY. Unfortunately, there remains no clear definition of antiplatelet 'resistance'. There is an accumulation of evidence, largely from studies performed *ex vivo*, that suggests significant inter-patient and temporal variability in response to these agents. In this article, *ex vivo* and clinical evidence regarding clopidogrel resistance is reviewed and potential mechanisms for this phenomenon summarised.

Ex-vivo Evaluation of the Antiplatelet Effects of Clopidogrel

A variety of techniques have been employed to quantify the variability in platelet inhibition associated with clopidogrel therapy. In these small studies, the percentage of patients found to exhibit at least some degree of resistance to clopidogrel varied dramatically with the type of assay, definition of resistance and the length of follow-up (see *Table 1*). Results from a recent trial serve to illustrate the difficulty in translating results from *ex*

vivo analyses to clinical practice. Lebarthe et al. measured the effect of clopidogrel therapy on platelet activation using optical platelet aggregometry in platelet-rich plasma, the current gold standard for such studies. Employing traditional methods and applying common criteria for clopidogrel resistance (<10% inhibition of peak platelet aggregation), a group of patients with stable angina were screened for resistance to clopidogrel. Thirty-five per cent of these patients were found to be non-responsive to clopidogrel after seven days of therapy; results comparable with those from other groups.⁸⁻¹⁰ In this study, the analysis was repeated with a more physiologic platelet agonist (r-hirudin and D-Phenylalanyl-prolyl-arginine chloro-methyl ketone), and the percentage of patients found to be non-responsive to clopidogrel fell to 12%. Furthermore, it has been argued that focusing on peak platelet aggregation (one minute after addition of the agonist) is less physiologic than measuring 'late' platelet aggregation (six minutes after the addition of the agonist).¹¹ When the analysis was repeated using the more physiologic agonist and late aggregation, the number of non-responsive patients fell to 6%, more consistent with recurrent event rates in clinical trials.⁸

Clinical Evidence for Clopidogrel Resistance

Ex vivo measurements of platelet activity have traditionally been difficult to relate to clinical events.¹² There are two small trials examining clinical outcomes in the setting of an inadequate response to clopidogrel. Matetzky et al. evaluated response to clopidogrel therapy among 60 patients undergoing percutaneous coronary intervention (PCI) in the setting of ST segment elevation MI. Platelet activity was assessed before and after clopidogrel administration via optical aggregometry, and by a cone and platelet analyser. In the six months following enrollment, there were eight cardiovascular (CV) events that occurred in seven patients. Seven of these events (88%) occurred among patients described as clopidogrel non-responders.¹³

In a larger study, Gurbel et al. randomised 120 patients scheduled to undergo elective PCI to



Table 1: Summary of Selected Clinical Trials Evaluating the Variability in Response to Clopidogrel

Trial	N (clinical setting)	Methodology	Definition of non-response	Prevalence of non-responders or adverse outcomes
Jeremo et al. ²⁷	18 (elective PCI)	Platelet bound fibrinogen	>10% residual platelet activity	60% at 24 hours
Lau et al. ²²	57 (elective PCI and healthy volunteers)	Platelet aggregometry in (PRP) and Plateletworks®	<10% platelet inhibition	22% at five days
Muller et al. ¹⁵	105 (elective PCI)	Platelet aggregometry In PRP	<10 % platelet inhibition	5% at four hours
Gurbel and Bliden ¹⁰	63 (elective PCI)	Platelet aggregometry in PRP	<10% platelet inhibition	37% at 24 hours
Gurbel et al. ⁹	96 (elective PCI)	Platelet aggregometry in PRP	<10% platelet inhibition	31% at 24 hours
Abeil et al. ²⁸	114 (with coronary disease and healthy volunteers)	VASP phosphorylation	Platelet reactivity identical to non-treated patients	>30%
Serebruany et al. ²⁹	544 (coronary disease or heart failure and healthy volunteers)	Platelet aggregometry in PRP	Two standard deviation below the mean response	4.2%
Labearthe et al. ⁸	16 (stable coronary disease)	Platelet aggregometry in PRP	<10 % platelet inhibition	35% at seven days
Matetzky et al. ¹³	60 (NSTEMI and PCI)	Platelet aggregometry in PRP	Lowest two quartiles of platelet aggregation	13% MACE all among patients in lower quartiles
Gurbel et al. ¹⁴	120 (elective PCI)	Platelet aggregometry in PRP	<50% platelet aggregation following therapy	3.3% rate of post-procedure MI

MACE = Major adverse cardiac events; NSTEMI = Non-serotonin elevation myocardial infarction; PRP = Platelet-rich plasma; VASP = Vasodilator-stimulated phosphoprotein.

treatment with 300mg of clopidogrel, 600mg, or the same doses in patients also receiving the glycoprotein (GP) IIb/IIIa inhibitor eptifibatide. Using the *ex vivo* measurements of platelet activity, platelet inhibition was more complete among patients treated with higher doses of clopidogrel and higher still among patients randomised to receive adjuvant GP IIb/IIIa inhibition. Most importantly, patients receiving more intensive antiplatelet therapy were less likely to exhibit post-procedural elevation in serum troponin levels. Owing to the fact that baseline platelet activity was related to responsiveness to clopidogrel, it was recommended that complete platelet inhibition should become a 'therapeutic target' in PCI.¹⁴

Potential Mechanisms for Clopidogrel Resistance

Numerous mechanisms could explain the observed variability in response to clopidogrel (see Table 2). Gurbel et al. noted that elevated baseline platelet activity was related to an inhibited response to clopidogrel in one study and increased post-procedural MI in another.^{9,14} Variability in the serum concentrations of compounds known to modulate platelet activity, such as adenosine diphosphate (ADP), nitric oxide, thrombin, tissue factor, or thromboxane A₂, may explain an inadequate response to clopidogrel. Muller et al. found that clopidogrel did not inhibit platelet aggregation in response to thrombin-related activating peptide, suggesting that elevated serum thrombin levels could overwhelm the effects of thienopyridines.¹⁵ Response to clopidogrel may also be related to bioavailability. Significant correlations between the inhibition of ADP-induced

platelet aggregation and plasma levels of clopidogrel, its active metabolite and its inactive carboxyl metabolite have been identified among 10 healthy volunteers.¹⁶ Higher doses of clopidogrel have been shown to be more effective in achieving complete inhibition of platelet activity, perhaps by providing higher concentrations of the active metabolite.¹⁴

Genetic polymorphisms of many proteins may modulate response to thienopyridine therapy. In particular, variation in the density of the platelet receptor that binds clopidogrel (the P2Y₁₂ receptor), or genetic polymorphisms in the receptor itself, could affect the response to clopidogrel. Fontana et al. have described variations in the structure of the P2Y₁₂ receptor that were associated with higher maximal platelet aggregation and more commonly found among patients with peripheral arterial disease.^{17,18} Other groups have characterised P2Y₁₂ receptor polymorphisms but have not consistently demonstrated increased platelet aggregation or a relationship to adverse events.^{19,20}

An important potential mechanism contributing to a lack of uniformity in response to clopidogrel is variability in cytochrome P450 3A4 activity. Cytochrome P450 oxidation is central to the generation of the active metabolite of clopidogrel. Lau et al. investigated this hypothesis in a study of 35 healthy patients and 35 patients undergoing elective PCI. Platelet aggregation studies were performed before and after treatment with clopidogrel, and cytochrome P450 3A4 activity was measured using the erythromycin breath test. A highly significant inverse correlation was found

Table 2: Potential Mechanisms for Clopidogrel Failure

Decreased plasma drug concentration due to decreased GI absorption
Decreased plasma drug concentration due to increased body weight, volume of distribution, etc.
Drug non-compliance
Decreased hepatic metabolism to active metabolite due to reduced cytochrome P450 3A4 activity or genetic polymorphisms in the P450 system
Decreased hepatic metabolism to active metabolite due to competition from other drugs for cytochrome P450 3A4 binding
Decreased density of P2Y ₁₂ receptors on the platelet surface
Polymorphisms in the P2Y ₁₂ receptor that reduce its affinity for clopidogrel
Increased local or systemic concentrations of other platelet activators (i.e. variation in baseline platelet activity)

GI=gastrointestinal.

between cytochrome P450 activity and clopidogrel non-responsiveness in healthy volunteers and those undergoing PCI. In addition, co-administration of rifampin, an inducer of the cytochrome P450 3A4 isoenzyme, and clopidogrel resulted in improved inhibition of platelet aggregation in a group of healthy volunteers.²¹

Additional evidence in favour of this mechanism was put forward by Matetzky et al., who noted that patients resistant to clopidogrel were less likely to be cigarette smokers than patients who responded as expected. The authors noted that nicotine and the aromatic hydrocarbons found in cigarette smoke activate the cytochrome P450 isoenzymes.¹³ Furthermore, in a separate study, Lau et al. demonstrated *in vitro* that atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation, via competition for cytochrome P450 3A4 isoenzyme binding.²² On the other hand, in the Interaction of Atorvastatin and Clopidogrel Study

(Interaction Study) no interaction between the antiplatelet effects of clopidogrel and statin therapy was identified using a variety of *ex vivo* methods to characterise platelet activity.²³ Perhaps most importantly, no evidence for increased risk of adverse events was detected among patients treated with statins and clopidogrel in retrospective analyses of data from several clinical trials.^{24–26} Most notably, in the Clopidogrel for the Reduction of Events During Observation (CREDO) trial (previously published as part of MedscapeCREDO trial) Saw et al. found no increase in the event rate among patients enrolled in CREDO concomitantly treated with CYP3A4-metabolised statins (i.e. atorvastatin) and clopidogrel.²⁵

Clopidogrel Resistance

The term ‘clopidogrel resistance’ has been used to describe patients who demonstrate a relatively poor response to clopidogrel *ex vivo*. It appears there are a significant number of patients who fail to respond to clopidogrel as expected. Additionally, there is evidence that a failure to respond appropriately is associated with an increased risk of adverse clinical events. Several of the proposed mechanisms for clopidogrel are reasonable and would predict and explain the wide inter-patient variability noted in some trials of *ex vivo* platelet response. However, whether use of the term clopidogrel resistance is appropriate deserves further study and consideration. Large-scale studies that carefully correlate baseline platelet activity with the degree of inhibition by aspirin and/or clopidogrel and with clinical events are necessary to validate the concept of antiplatelet resistance and shed light on the mechanism(s) involved. ■

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