Coming safely to a stop: A review of platelet activity after cessation of antiplatelet drugs

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Abbreviations:

ADP adenosine diphosphate
DAPT dual antiplatelet therapy
NSTEMI non-ST elevation myocardial infarction
PCI percutaneous coronary intervention
RCT randomised controlled trial
STEMI ST-elevation myocardial infarction

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Abstract

The platelet P2Y12 antagonists are widely used, usually in combination with aspirin, to prevent atherothrombotic events in patients with acute coronary syndromes, during PCI and after placement of arterial stents.

Inhibition by clopidogrel or prasugrel lasts for the lifetime of the affected platelets and platelet haemostatic function gradually recovers after stopping the drug, as new unaffected platelets are formed.

The optimal durations for dual antiplatelet therapy are prescribed by clinical guidelines. Continuation beyond the recommended duration is associated with an increased mortality, mainly associated with major bleeding.

Fear of a “rebound” of prothrombotic platelet activity on stopping the drug has provoked much discussion and many studies. However, review of the available literature reveals no evidence for production of hyper-reactive platelets after cessation of clopidogrel in stable patients. Any increase in acute coronary and other vascular events after stopping seems most likely therefore to be due to premature discontinuation or disruption of treatment while thrombotic risk is still high. No difference in rebound was found with the newer P2Y12 inhibitors, although ticagrelor and prasugrel are more potent platelet inhibitors than clopidogrel.

Recent RCTs confirm it is safe to stop the thienopyridine and continue with aspirin alone in most patients after the duration of treatment recommended by the guidelines. Decisions on when to stop therapy in individuals, however, remain challenging and there is a growing rationale for platelet testing to assist clinical judgement in certain situations such as patients stopping DAPT before surgery or in individuals at highest bleeding or thrombotic risk.
**Background**

Medical therapy to reduce platelet activation is a mainstay in prevention of atherothrombotic events in cardiovascular disease. For many years aspirin was the drug of choice, but randomised controlled trials from the 1990s onwards demonstrated the efficacy of clopidogrel in reduction of major acute coronary and cerebrovascular events and vascular deaths (CAPRIE 1996). Clopidogrel in combination with aspirin improved outcomes after acute coronary syndrome, and after percutaneous coronary intervention (PCI) with or without placement of stents (Bhatt et al 2006, Chen et al 2005; Steinhubl et al 2003; Yusuf et al 2001).

Two newer P2Y12 inhibitors are in clinical use. Prasugrel, another irreversible thienopyridine inhibitor (Wiviott et al 2007), and ticagrelor, a reversible, non-competitive inhibitor (Wallentin et al 2009), are now preferred over clopidogrel for acute myocardial infarction for their more rapid and more potent antiplatelet action (Windecker et al 2014). However, the association with higher bleeding risk, contra-indication in certain categories of patient, and higher cost, mean that clopidogrel is still widely-used throughout the world, and following PCI in stable coronary artery disease. For the purposes of this review I will concentrate on clopidogrel.

**Action of Clopidogrel**

Clopidogrel is a prodrug of the thienopyridine class. Conversion to the active metabolite in the liver is dependent on cytochrome P450 isoforms including CYP3A and CYP2C19. There is well-recognised variability in responsiveness to clopidogrel, which is at least partly attributable to polymorphisms of these genes (Simon et al NEJM 2009), as well as to drug interactions and adherence to treatment. The main therapeutic target for the active molecule is the platelet P2Y12 receptor for adenosine disphosphate (ADP). Through a Gi-coupled signalling pathway, receptor occupancy induces activation of the alpha IIb beta 3 integrin receptor (also known as Glycoprotein IIb-IIIa), exposing the receptor for fibrinogen and resulting in platelet aggregate formation (Braun et al 2007; Schror 1998). Inhibition of the P2Y12 is irreversible and so, as with aspirin blockade of cyclo-oxygenase, this pathway of activation is inhibited for the lifetime of the platelets (Schror 1998). Function gradually...
recovers after stopping the drug because of new, unaffected platelets being produced from megakaryocytes, while ageing platelets are gradually removed. Normal platelet lifespan is 7-10 days, but disease and ageing may influence turnover times and therefore affect the time to recovery of platelet function after a single antiplatelet dose.

**Duration of Dual Antiplatelet Therapy**

Evidence-based, national and international clinical guidelines prescribe the optimum durations for antiplatelet therapy, and these have evolved considerably over the past decade as more trial results emerge (Jneid et al 2012; Windecker et al 2014). The European Society of Cardiology (Windecker et al 2014) currently states that after acute myocardial infarction – STEMI or NSTEMI, dual antiplatelet therapy (DAPT) with aspirin plus a P2Y12 inhibitor be continued for 12 months after the acute event; whereas for patients with stable coronary artery disease who receive PCI and a drug-eluting stent, clopidogrel is recommended for 6 months, and for a minimum of one month after placement of a bare metal stent. It is usually recommended that patients continue on aspirin for life, unless contraindicated, for example by drug intolerance or bleeding.

Because of the pivotal role of platelets in haemostasis and thrombosis, maintenance of the balance between inhibition and activation is essential. Thus, all antithrombotic agents introduced to date carry the side-effect of increased bleeding risk. As the thrombotic risk diminishes over time, for example after successful PCI to unblock the diseased coronary artery, or healing of the vessel wall after placement of stents, the risk of mortality from bleeding becomes disproportionally higher with continued use of DAPT. However, the European guidelines (Windecker et al 2014) also state that a longer duration can be used in those at high ischaemic risk and low bleeding risk, and a shorter duration in people at lower risk of recurrence or higher bleeding risk. In addition, the need may arise to withdraw antiplatelet drugs earlier than recommended, before surgery for example, or if there is intolerance to the drug, or bleeding events.

**What happens clinically on stopping clopidogrel?**

The publication of a series of case-reports and retrospective studies sounded the alarm over an apparently increased rate of ischaemic events in the period after withdrawal of clopidogrel.
Stopping antiplatelet drugs

(Ong et al 2005; Pfisterer et al 2006; Ho et al 2008; Ho et al 2010). Ho et al (2008) reported a clustering of adverse events in patients with previous acute coronary syndrome in the first 90 days after stopping clopidogrel. Despite the observational and retrospective nature of these studies, an “Advisory” statement was issued on premature stopping of DAPT (Grines et al 2007) (see Gaglia and Waksman 2011 for systematic review).

What happens to platelets when clopidogrel is withdrawn?

These studies provoked discussion of a “rebound” in platelet activity. The theory was proposed that suppression by clopidogrel might lead to a biological adaptation of platelets or megakaryocytes, conferring enhanced sensitivity to ADP or other stimuli. Such a change would manifest itself after cessation of the drug and recovery of a non-inhibited, hyperactive platelet population (Lordkipanidze et al 2009; Sambu et al 2011). This gave rise to some small studies that claimed to find a rebound of platelet responsiveness at varying times after stopping clopidogrel treatment.

An observational study of 28 patients with coronary stent implantation, who had been treated with clopidogrel for 12 months, found that ADP-stimulated platelet aggregation was increased at two weeks after withdrawal of clopidogrel, compared to on-treatment levels, and remained elevated at six weeks, as would be expected [Diehl et al]. However on testing at 17 weeks after stopping, aggregation was lower than at the earlier time-points, and was now no different from that in a group of untreated stable CAD controls.

Mylotte et al. (2011) studied 32 patients with drug-eluting stents (DES). Between on-treatment values and the sample taken at one week following withdrawal, there was an increase in aggregation response to a range of doses of ADP, epinephrine, and thrombin-receptor-activating peptide (TRAP), but not arachidonic acid (AA). There was a further increase in aggregation induced by the lower, but not higher, doses of ADP at one month. By three months after cessation however, aggregation with ADP and epinephrine had decreased, returning to levels similar to those found at seven days, and this was interpreted as a transient rebound effect occurring at one month post-cessation.

The prospective CESSATION study enrolled 28 patients who stopped clopidogrel therapy one year following insertion of a DES. Using the more unusual technique of
thromboelastography platelet mapping (Short TEG), there was gradual recovery of ADP-induced aggregation up to one week, but levels remained similar at two and four weeks, and so no evidence was found for a rebound in ADP pathways of aggregation (Sambu et al 2011b).

Thirty-seven patients with clinically stable coronary artery disease who had been taking clopidogrel and aspirin (for varying lengths of time) were compared with a control group of stable CAD patients who were not taking clopidogrel (Lordkipanidze et al 2014). There were no differences in the Verify Now P2Y12 test, but there was a difference in ADP-stimulated aggregation between the clopidogrel and control groups at seven and 28 days after stopping. Difficulties in comparing these two groups directly include the considerable individual variability in platelet response, and the reasons for being prescribed clopidogrel or not, which most likely include severity of arterial disease and the estimated thrombotic and bleeding risk.

On the other hand, in a study of 200 patients who had taken clopidogrel for 12 months after stenting, there was no evidence for rebound [Djukanovic 2011]. Although again there was no pre-treatment test, platelet aggregation by a whole blood multi-electrode impedance assay (Multiplate®) merely reached a plateau around ten days after stopping and was not significantly different at 45 or 90 days.

However, as none of these studies included a pre-treatment baseline measurement, it was not possible to say whether platelet responsiveness was merely returning to pre-treatment levels.

**Platelet studies with a pre-treatment baseline**

Several prospective studies have been performed that included detailed platelet function analysis at a pre-treatment baseline as well as on-treatment, and with follow-up for a least a month after stopping clopidogrel. In a placebo-controlled cross-over study of 15 healthy subjects who took clopidogrel plus aspirin or placebo plus aspirin for 7 days each, a comprehensive range of platelet measurements was made. These comprised activation markers GPIIb/IIIa and P-selectin by flow cytometry, aggregometry in platelet-rich plasma and in whole blood, all with a range of doses of ADP and other agonists; counting of the immature platelet fraction, and soluble CD40Ligand assay. Once platelet function had
recovered - by around eight days after stopping - there was no further increase in stimulated platelet aggregation or activation markers and no significant differences in any of the tests at 11, 15 or 45 days post cessation compared to baseline [Frelinger et al 2010]. These data showed conclusively that a short-term course of clopidogrel did not increase regeneration nor alter the reactivity of newly-produced platelets, at least in healthy subjects.

In a prospective, double-blind placebo controlled trial, Ford et al (2014) randomised 171 patients with stable coronary artery disease or peripheral arterial disease to 28 days of clopidogrel (75mg) or placebo. Patients continued to take their 75mg daily aspirin therapy throughout the study. A range of platelet tests was carried out at pre-treatment baseline, on-treatment (just before stopping clopidogrel or placebo), and at seven, 14 and 28 days after stopping. ADP-stimulated platelet aggregation (5 and 10 micromolar ADP), platelet activation markers by flow cytometry, Verify NowP2Y12 and VASP-P, were all significantly lower on clopidogrel treatment compared with baseline, showing appropriate responses to the drug. Unstimulated markers of platelet activation were unchanged. Values returned to baseline levels by seven days after discontinuation. Apart from the on-treatment measurements, there were no statistically significant differences between those taking clopidogrel or placebo at any of the follow-up timepoints. Furthermore, there was no evidence for a treatment-time interaction in any of the statistical models, thereby confirming that platelet responsiveness remained stable over time after stopping clopidogrel (Ford et al 2014).

Two studies used the approach of comparing the recovery of platelet activity in patients who were allocated to abrupt cessation of clopidogrel or to a tapering regimen at the planned completion of their post-stent treatment, with the aim of attenuating any rebound. Neither study found any evidence for a difference in platelet activity after complete cessation (Sibbing et al 2010; Yedidya et al 2012).

In summary, in studies with adequate numbers, baseline pre-treatment measurements and inclusion of a placebo arm, therefore, there has been no evidence for a rebound of platelet responsiveness to ADP after stopping clopidogrel. Instead, these studies show a gradual return to original levels of platelet activation and responsiveness. It is unlikely that any rebound effect would be missed in a 30-day follow-up as this is more than adequate for a complete turnover of the platelet population. Furthermore, most of the reported excess of thrombotic events occurs within the first 30 days of stopping (Lemesle et al 2011). It has also
been argued that increased sensitivity of the P2Y12 receptor may develop over longer-term therapy. However, the incidence of post-clopidogrel thrombotic events is highest in patients who discontinued the drug within one month of commencing [Lemesle et al 2011], a finding more consistent with discontinuation before healing of the arteries was complete.

Most of the controlled laboratory studies selected patients with stable coronary artery disease, and it could be argued that a greater effect might be seen in patients with ACS or drug-eluting stents as they are at higher thrombotic risk. Patients with acute events would unfortunately be more difficult to study rigorously because of the expected long interval between pre-treatment sample and stopping clopidogrel. Caution should of course be exercised in extrapolating the results to patients with a recent acute event.

**Could there be adaptation of other platelet pathways to clopidogrel?**

Reflecting the vital function of haemostasis and thrombus formation, platelets carry many different surface receptors that recognise various stimulatory agonists, and several signalling pathways for activation. As well as the established target of P2Y12, it is apparent that clopidogrel may inhibit aggregation stimulated by other agonists, including arachidonic acid and TRAP (Ford et al 2013). Some studies have found that, although there was no rebound effect on ADP pathways of activation or aggregation, the “aspirin-specific” tests, i.e. using arachidonic acid as platelet stimulus, appeared less inhibited after stopping clopidogrel but maintaining the aspirin dose (Djukanovic et al 2014; Hobson et al 2009, Sambu et al 2011). However, detection appears to be method-dependent, as it is not seen with light transmission aggregometry (Ford et al 2013, Ford et al 2014, Frelinger et al 2010), and Good et al [2015] have recently shown that there is no additional suppression of thromboxane B2 production when clopidogrel is added to aspirin therapy. Nevertheless, such synergistic effects probably contribute to the efficacy of dual compared with single antiplatelet therapy.

Reactive inflammation following cessation of clopidogrel has also been proffered as an explanation for an increase in MACE (Angiolillo et al 2006). The results are conflicting however and are likely to reflect changes in disease state rather than an association with stopping clopidogrel (Sambu et al 2011; Wykrzykowska et al 2009). It is unclear in any case whether clopidogrel therapy directly affects any inflammatory markers although, in common
with aspirin, appearing to be associated with lower C-Reactive Protein (CRP) (Woodward et al. 2004; Husted et al. 2010).

Despite lacking a nucleus, platelets contain the translational mechanisms needed to manufacture proteins (Weyrich et al 1998), and so an adaptation response of either megakaryocytes or platelets to chronic antiplatelet therapy is not implausible (Lordkipanidze 2009). However there is no evidence to support any pro-thrombotic changes in receptor number or signalling capacity of platelets in the weeks after antiplatelet therapy in humans, and in any case this would not explain why any such changes were not detectable by the range of functional tests employed in the controlled research studies.

**Newer P2Y12 Inhibitors and Platelet Activity**

Ticagrelor (a reversible inhibitor) and prasugrel induce more potent inhibition of platelet function than clopidogrel (Storey et al 2011) and, in the case of prasugrel, a more delayed recovery of platelet function after stopping (Price et al 2012). Studies so far have found no sign of rebound on stopping either prasugrel or ticagrelor (Angiolillo et al 2011; Gurbel et al 2009; Gurbel et al 2011; Jakubowski et al 2011), and as their modes of action are similar this would seem rational. Reversible inhibitors of P2Y12 allow more rapid return of haemostatic activity when stopped. These would be considered to be beneficial properties if urgent surgery or intervention were required, or if bleeding problems arose. On the other hand, issues of poor compliance could be more serious as a missed dose would produce intermittent reversal of platelet inhibition.

**Is there a true clinical rebound?**

A systematic review (Gaglia and Waksman 2011) found most early studies to be flawed, and more recent detailed analyses have cast doubt on the existence of a clinical rebound particular to clopidogrel. A retrospective analysis (Collet et al 2009) of the CHARISMA trial data (Bhatt et al 2006) found that in almost 3000 patients who stopped taking medication before the end of the study, there were increased rates of ischaemic events and overall mortality, but the rate was actually lower in the clopidogrel plus aspirin group compared with the placebo plus aspirin group (Collet et al 2009). Bleeding events were higher than in those who
completed, but no different between those who had been taking clopidogrel or placebo, and
the investigators concluded there was no evidence for a clinically-detectable rebound.

Controversy still remains over the ideal duration, but the data now emerging from
randomised trials demonstrate that extending the recommended time might be of no overall
benefit and even detrimental (El Hayek et al 2014; Mauri et al 2014; Schulz-Schupke et al
2015;Valgimigli et al 2013). In the DAPT Trial (Mauri et al 2014), the risk of stent
thrombosis was reduced but all-cause mortality and the incidence of severe or moderate
bleeding were significantly higher in the group that continued a thienopyridine to 30 months.
Interestingly the rate of stent thrombosis increased in the three months after stopping in both
the group that continued on clopidogrel and the group that had changed to placebo.

**Are the Reasons for Stopping Antiplatelet Drugs Important?**

In most studies, the timing and the reasons for stopping antiplatelet therapy were unknown.
The PARIS registry study (patterns of non-adherence to antiplatelet regimens in stented
patients) (Mehran et al 2013) followed patients for two years and analysed the outcomes
according to pre-specified reasons for stopping:

1) “discontinuation”, defined as physician-recommended cessation;
2) “interruption”, defined as temporary cessation of DAPT of less than 14 days due to
   surgical procedures;
3) “disruption”, defined as withdrawal of antiplatelet treatment due to bleeding or non-
   compliance.

In 5031 patients, the rate of DAPT cessation by 24 months was 57.3%, mostly as physician-
recommended discontinuation (40.8%), with disruption in 14.4% and interruption in 10.5%.
Three-quarters of all events occurred in patients while still taking DAPT, but compared with
those on treatment, the interruption and disruption group had a higher risk of major acute
coronary events (MACE). In contrast, those with physician-recommended cessation had
lower MACE risk than those who remained on DAPT at 24 months. The highest risk ratio in
the disruption group occurred in the first 7 days after stopping. This is consistent with the
study of Lemesle et al (2011) who found that those stopping earliest had the highest risk of
death, MI, and stent thrombosis at 30 days.
The evidence gives reassurance that discontinuation of DAPT in low-risk patients should be considered safe, especially after 6 months from stent implantation (Franchi and Angiolillo 2014). Unanticipated premature disruption of treatment however is likely to place the patient at higher risk. Patients in this latter group are more likely to have stopped because of drug intolerance, bleeding issues or illness. Although there is no sign that clopidogrel leads to production of hyper-reactive platelets, after drug withdrawal uninhibited platelets in circulation will become activated by encountering areas of atherosclerotic vessel wall, or by increased shear stresses in narrowed arteries, or by the prothrombotic stimulus of unhealed stents.

**The Future of Platelet Testing to Assess Risk and Guide Therapy**

Determining the right time to stop antiplatelet therapy in individuals remains a major issue for clinical judgement. The case for routine platelet testing at the time of PCI to guide therapy has not been supported by the recent GRAVITAS and TRIGGER-PCI trials (Price et al 2011; Trenk et al 2012), although in the study of Aradi et al (2015), switching those with highest on-treatment platelet reactivity from clopidogrel to prasugrel appears more promising. Testing to stratify those patients at higher risk for either bleeding or thrombotic events could be more useful (Collet and Montalesco 2013) and there is proven value in specific situations, such as when surgery is required.

In the perioperative period, decision making on interruption of DAPT and removal of cardioprotection is complicated by the increase in both bleeding risk and hypercoagulability. (Patel and Fleisher 2014). Where it is deemed safe to do so, discontinuation of P2Y12 inhibitors five days before cardiac surgery is advised to allow recovery of platelet function, although it is generally accepted that aspirin can be continued (Ferraris et al 2012). Marked individual variability in return of adequate haemostatic activity is evident however (diDedda et al 2014) and time to recovery is determined, inter alia, by the level of platelet reactivity before drug exposure and the extent of platelet inhibition immediately after discontinuation (Price et al 2012). A strong rationale is emerging for platelet testing; particularly suitable are the rapid whole blood methods requiring minimal sample preparation, such as the Multiplate (multi-electrode impedance aggregometry) and the point of care test, Verify Now (Reviewed by Petricevic et al 2015, Kong et al 2014). In addition, in cases where surgery cannot be delayed, testing may be used to predict the need for blood products (Emeklibas et al 2013).
Conclusion

“Drug discontinuation effects are part of the pharmacology of a drug” (Reidenberg 2011) and as such are an important part of safety evaluation. The term “rebound” has been applied to denote an unexpected increase in adverse clinical events at the end of a course of treatment, and has been applied to a range of drugs (Graves et al 1997). There has also been some misuse of the term “rebound” in the literature to denote simply a return to the status quo of the pre-drug state. The current evidence indicates that there is nothing unusual about clopidogrel in this regard: platelet responsiveness gradually recovers to the pre-drug levels after stopping. The most likely explanation for post-clopidogrel ischaemic events is premature withdrawal of required protection while the damaged vessels continue to present a highly prothrombotic stimulus. A role exists for platelet testing to assist in clinical decision making.
References


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