

Antiplatelet Drug's Resistance

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Abstract: Antiplatelet therapy, targeting the inhibition of platelet function, ameliorates the survival of patients with clinically evident cardiovascular disease. The cornerstone of therapy is aspirin which was found to reduce of about 20% the relative risk of death, myocardial infarction and ischemic stroke. Clopidogrel in combination with aspirin is the recommended standard of care for reducing the occurrence of cardiovascular events in patients presenting with acute coronary syndromes undergoing percutaneous coronary intervention (PCI). Despite the benefits of current antiplatelet therapies, there are significant shortcomings, including hyporesponsiveness, delayed platelet inhibition, individual variability in response, a prolonged time to recovery. In addition, a growing body of evidence is demonstrating that a residual platelet reactivity on antiplatelets is associated with a significant increased risk of adverse clinical events. The optimal level of platelet inhibition to prevent cardiovascular events is modulated by clinical situation and at the moment we do not have a platelet function cut-off which is widely accepted to identify patients at high risk for ischemic events in the different clinical models. Ongoing clinical trials are evaluating if an antiplatelet treatment tailored on the entity of platelet inhibition will be a good strategy in terms of safety and efficacy. These studies will give us the crucial information on the possible utility of a monitoring of antiplatelets in order to prevent clinical events.

INTRODUCTION

Large clinical trials have shown that dual antiplatelet therapy (aspirin plus clopidogrel) reduce the risk of recurrent cardiovascular events in patients with coronary artery disease. Dual antiplatelet therapy is the standard care in patients with acute coronary syndromes [1-5].

Aspirin irreversibly acetylates serine residue (ser529) in COX-1 preventing the binding of arachidonic acid to the catalytic site. Controversy exists regarding the clinical relevance of non-COX-1 mediated antiplatelet effects of aspirin [6]. Clopidogrel is a second-generation thienopyridine that is converted to an active metabolite by the hepatic cytochrome P450 pathway. The active thiol metabolite of clopidogrel forms a covalent disulfide bond with cys17 and cys270 residues present in the extracellular domains of P2Y12 and inhibits ADP binding [6].

New P2Y12 receptor antagonists are currently undergoing investigation. Prasugrel is a third generation thienopyridine that is associated with greater active metabolite generation, superior inhibition of ADP-induced platelet aggregation and less response variability than clopidogrel [7]. In the Trial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitionN with prasugrel (TRITON-TIMI 38 trial), prasugrel was compared to clopidogrel in patients with moderate to high risk acute coronary syndromes undergoing PCI [8]. The prevalence of cardiovascular death, nonfatal myocardial infarction or non-fatal stroke was lower with prasugrel treatment compared to clopidogrel (12.1% vs 9.9%). However, there were higher rates of bleeding in the prasugrel group. TRITON conclusively demonstrated that superior P2Y12 blockade produces superior reduction in ischemic events in acute coronary syndromes.

RESIDUAL PLATELET REACTIVITY ON ANTI-PLATELET THERAPY: BEYOND THE CONCEPT OF 'RESISTANCE'

The issue of the optimal dosage of clopidogrel – and aspirin – to reduce the ischemic events is linked to the issue of the so-called 'resistance' to antiplatelets. It has been clearly demonstrated that there is a great variability in the entity of inhibition of platelet function induced by these drugs. Pharmacologists define 'resistance' to a drug on the basis of the measurement of the metabolite which is the specific target of that drug: cAMP for clopidogrel and thromboxane for aspirin. On the other hand, clinicians are interested in the evaluation of how the entity of platelet function inhibition induced by antiplatelets affects the risk of recurrent ischemic events. Therefore we believe it is crucial to overcome the concept of 'resistance' with that of 'residual platelet reactivity' on antiplatelet treatment. A growing body of evidence is linking the entity of platelet inhibition on therapy with cardiovascular recurrences. The optimal level of platelet inhibition to prevent cardiovascular events is modulated by clinical situation and at the moment we do not have a platelet function cut-off which is widely accepted to identify patients at high risk for ischemic events in the different clinical models. Ongoing clinical trials are evaluating if a antiplatelet treatment tailored on the entity of platelet inhibition will be a good strategy in terms of safety and efficacy. These studies will give us the crucial information on the possible utility of a monitoring of antiplatelets in order to prevent clinical events.

Multiple factors have been proposed to explain individual variability of response to antiplatelet drugs. As with many medications, noncompliance is a consideration. Poor absorption could contribute to a decreased response to clopidogrel and aspirin, as Michelson proposed [9]. Upregulation of platelet production may overcome the irreversible mechanism of the antiplatelet medications [9].

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Laboratory methods that were used to evaluate platelet response included light transmission aggregometry (LTA), flow cytometry, PFA-100 (platelet function analyzer), the Ultegra Rapid Platelet Function Assay- VerifyNowASA and VerifyNowP2Y12. The gold standard for evaluating platelet responsiveness is LTA. However, aggregometry is time-consuming and requires a specialized laboratory. Flow cytometry by use of the vasodilator-stimulator phosphoprotein (VASP) assay detects the availability of the P2Y12 receptor and determines the effectiveness of clopidogrel. The point-of-care tests are less laborious and more time-efficient. The PFA-100 uses cartridges with epinephrine or adenosine-diphosphate (ADP) to mimic a high-shear state to evaluate platelet function. The VerifyNow assay system is a turbidimetry-based optical device that measures platelet aggregation. We compared the VerifyNowP2Y12, PFA-100, VASP and LTA and we found a significant correlation between LTA, VASP and VerifyNow [10,11].

ASPIRIN

Aspirin irreversibly acetylates serine residue (ser529) in COX-1 preventing the binding of arachidonic acid to the catalytic site, which prevents the formation of thromboxane A₂, a platelet activator [6]. Aspirin could also interfere with platelet function by impairing neutrophil-mediated platelet activation [6].

Clinical trials show that women are more likely to be aspirin-resistant than men [12-16]. We and others found that advanced age, diabetes and reduced left systolic ventricular function were all associated with a significant higher risk to have a residual platelet reactivity on treatment (measured by both light transmission aggregometry induced by arachidonic acid and by PFA-100) [16, 17]. Advancing age may cause a decrease in metabolism, which could predispose the elderly to underutilization of aspirin. Diabetes and reduced left systolic ventricular function are conditions associated with higher platelet reactivity which might account for the reduced response to the drug. Finally, we have documented that the inflammatory state is associated with the entity of platelet inhibition by aspirin [18].

Clinical studies have indicated that patients who are resistant to aspirin can be at risk of developing clinical recurrences. Gum *et al.* found that aspirin-resistant patients faced an increased risk of stroke, myocardial infarction and vascular death [15]. Eikelboom and colleagues reported that elevated levels of urinary-11 dehydrothromboxane B₂ (a degradation product of thromboxane A₂) predicted future myocardial infarction or cardiovascular death both in the patients of HOPE trial [19] and CHARISMA [20]. Grottemeyer and co-authors reported that 40% of aspirin non-responders experienced subsequent stroke, myocardial infarction or vascular death [21]. Mueller studied 100 patients who underwent balloon angioplasty and found that 87% who failed to show inhibition to aggregation experienced reocclusion of the treated lesion within 18 months [22]. We found that acute coronary syndromes patients with residual platelet reactivity on aspirin treatment have a significant increased risk of major adverse cardiovascular events at a 12 month follow-up [23, 24].

CLOPIDOGREL

Clopidogrel is a prodrug that is metabolized by cytochrome P450 into an active metabolite, which irreversibly inhibits binding of ADP to the P2Y₁₂ receptor on the platelet. Only 15% of the dose of clopidogrel absorbed is metabolized in the active drug, in particular by cytochrome P3A4 (CYP3A4) [6].

Concurrent medication use may interfere with the ability of clopidogrel to decrease platelet reactivity. Gurbel and colleagues reported that high doses of calcium-channel blockers and angiotensin-converting enzyme inhibitors possibly contribute to a decreased response to clopidogrel [25]. Atorvastatin is the most frequently lipophilic statin studied in clopidogrel trials as it has a high affinity for CYP3A4. Studies that have evaluated clopidogrel resistance and atorvastatin have not been uniformly reproducible, either. Lau *et al.* showed that atorvastatin promoted clopidogrel resistance at 10,20 and 40 mg [26]. On the contrary pravastatin, which has a non hepatic metabolism was found not to significantly affect platelet inhibition by clopidogrel. Subsequent studies, however, did not confirm this interaction. Post-hoc analysis of several important trials – among which CREDO, PRONTO and MITRA PLUS – have not found a significant interaction between statins (atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin) and clopidogrel in terms of entity of platelet inhibition [27, 28].

CYP2C9, CYP2C19 and CYP1A2 enzymes are involved in the conversion of clopidogrel to the active drug, too. It has been demonstrated a drug interaction mediated by CYP2C19 [29]. Gilard and colleagues reported that the contemporary assumption of clopidogrel and protonic pump inhibitor omeprazole, which binds CYP2C19, is associated with a lower level of inhibition of platelet function [30]. Further studies are needed to confirm this datum which has a particular clinical relevance as these drugs are often taken together in order to prevent gastrointestinal bleeding.

Tauber and colleagues demonstrated that the intestinal absorption is the main factor determining the production of active metabolites of clopidogrel [31]. In line with these results are those reported by ISAR-CHOICE: by comparing the effects of clopidogrel on the inhibition of platelet function at higher loading doses (300, 600 and 900 mg) it was demonstrated that there are no advantages in doses higher than 600 mg, as this does not result in a corresponding higher level of active metabolite in plasma [32]. This datum suggests that intestinal absorption is reduced for doses higher than 600 mg.

Also genetic polymorphisms have been investigated in order to evaluate a possible genetic determinant of residual platelet reactivity. Recently, an allelic variant of CYP2C19*2 (allele 681A) was found to be associated with an impaired platelet inhibition after clopidogrel administration in healthy subjects. In 1419 acute coronary syndrome patients on dual antiplatelet therapy, we found that allele 2C19*2 of CYP2C19 gene is an independent predictor of residual platelet reactivity and associated, independently from phenotype 'platelet reactivity', with the risk of adverse clinical events (stent thrombosis and cardiovascular deaths)

in the patients enrolled in the RECLOSE trial [33, 34]. Three papers contemporarily published in the literature demonstrated that this allelic variant is associated with the risk of recurrences [35-37].

We and others have found that diabetes, acute coronary syndrome, obesity and reduced systolic ventricular function are associated with a significantly higher prevalence of residual platelet reactivity on therapy [17]. Acute coronary syndrome is characterized by an enhanced platelet reactivity caused by a higher platelet turn-over, documented by the presence of reticulated platelets which are another determinant of the entity of platelet inhibition [38]. Diabetes and heart failure are known to be associated with a higher platelet reactivity too. Finally, inflammation, measured by white cells number, ESR and the balance between pro and anti-inflammatory cytokines, has been associated with diabetes, acute coronary syndrome and heart failure and it has been demonstrated to be a predictor of on-therapy platelet reactivity [17, 18].

Evidence suggests that poor responders to clopidogrel experience more frequent cardiovascular events than do responders. A limit in considering these results is that each study had different definitions for an individual's response to clopidogrel on the basis of the amount of agonist used, the assay used and the timing of the blood sampling. In 2007 it has been published the first meta-analysis on the data present in the literature, calculating the risk associated with a residual platelet reactivity on clopidogrel as an OR=8 (95% 3.4-19.1) on a total of 1025 patients undergoing PCI with stenting [39]. The significant heterogeneity is a limit of this analysis which reflects the variability in the methods and patients present in the published papers.

Since this meta-analysis, several studies have been published which, on a total number of 3271 patients, have all confirmed the association between residual platelet reactivity on clopidogrel and the enhanced risk of adverse clinical events. In particular, data from RECLOSE [40] trial showed that a reduced response to clopidogrel – measured by LTA induced by 10 micromol ADP – is an independent predictor of stent thrombosis and cardiovascular death in 804 patients undergoing PCI with implantation of a drug eluting stent. From a subsequent analysis of the same cohort of patients, we found that the contemporary reduced response to both aspirin (measured by LTA induced by arachidonic acid) and clopidogrel is the most important predictor of the same endpoints (stent thrombosis and cardiovascular death) [41]. This result underlines that a 'global' platelet hypereactivity identifies vulnerable patients at higher risk of recurrences. A great attention has been focused on the clinical validation of the point-of-care tests. Price *et al.* [42] showed, on 380 patients undergoing PCI with stent implantation, that residual platelet reactivity on clopidogrel measured by VerifyNow P2Y12 is associated with a significantly higher risk of adverse clinical events at a 6-month follow up. ARMYDA-PRO study [43] reported a significant association between residual platelet reactivity (measured by VerifyNow P2Y12) and 30-days MACE on 160 patients undergoing PCI. Finally, we demonstrated on a larger number of patients (683 acute coronary syndrome patients) that residual platelet reactivity measured

by VerifyNow P2Y12 is associated with a higher risk of cardiovascular death and nonfatal myocardial infarction at a 12-month follow up [44].

MANAGEMENT OF ANTIPLATELETS' DRUG RESISTANCE

There is, at present, little evidence to guide treatment of the patients with laboratory evidence of a residual platelet reactivity to antiplatelet drugs or thrombosis occurring during antiplatelet therapy. Empirical strategies include increasing the dose of the antiplatelet agent or adding a second antiplatelet drug.

Using laboratory assay, there is some evidence that aspirin response may be dose-dependent [45]. On the other hand, meta-analysis of the randomized clinical trials indicates that across the study populations, the most effective dose of aspirin with the fewest adverse consequences is 75-150 mg once daily [46]. It is possible that these large trials might include a small cohort of patients who would have benefit from a higher aspirin dosage. The response to aspirin may decline over time due to tachyphylaxis [47]. Andersen and colleagues found that 10% of non-responders to aspirin became responders at 4-month follow-up without a change in therapy [48]. These findings support the idea of a variable response and that resistance to aspirin can be overestimated depending on the timing of the laboratory evaluation. However, this could affect the durability of a 'hypothetic' treatment of residual platelet reactivity without reducing the importance of the results obtained by studies demonstrating that a single platelet function assessment identifies vulnerable and at-risk patients.

Similar trials have also shown a higher platelet inhibition with increased dosage of clopidogrel, combining synergistic medications and evaluating medications that potentially hinder the p450 conversion of clopidogrel into its active form. Gurbel and colleagues showed that loading with 600 mg of clopidogrel decreased platelet reactivity in comparison with 300 mg [49]. In a similar trial, von Beckerath evaluated different doses of clopidogrel in 60 patients who had ischemic heart disease undergoing elective PCI, and found that 600 mg of clopidogrel increased platelet inhibition over a 300-mg loading dose [32]. Loading doses greater than 600 mg of clopidogrel did not increase platelet inhibition, most likely due to limited absorption. In the OPTIMUS study [50], Angiolillo evaluated patients with type 2 diabetes mellitus and coronary artery disease and found that a 150 mg dose of clopidogrel is associated with a reduced platelet inhibition in patients with a residual platelet reactivity on standard therapy. None of these studies reported a significantly higher bleeding rate in patients on higher clopidogrel doses. Lau showed that clopidogrel given with rifampin, a cytochrome p450 P3A4 inducer, converted non responders to responders [51].

The ongoing trials, GRAVITAS and DANTE will establish whether an increase in the clopidogrel maintenance dose (150 mg vs 75 mg daily) is necessary in patients with residual platelet reactivity on clopidogrel, i.e. if a treatment tailored on the extent of platelet inhibition is associated with a reduced number of recurrences.

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