Antiplatelet therapy: does it need laboratory monitoring?

M. Cattaneo
Unità di Medicina 3 – Ospedale San Paolo. Dipartimento di Scienze della Salute.
Università degli Studi di Milano. Milano, Italy

The main antiplatelet agents that are currently used in clinical practice are acetylsalicylic acid (aspirin), which irreversibly inhibits the cyclooxygenase-1 (COX-1)-dependent production of thromboxane A\(_2\) (TxA\(_2\)), the thienopyridines, which irreversibly inhibit the platelet P2Y\(_{12}\) receptor for adenosine diphosphate (ADP), and inhibitors of the glycoprotein complex IIb/IIIa (integrin \(\alpha_{IIb}\beta_3\)).

In the last few years, several studies revealed interpatient response variability to aspirin and the thienopyridine drug clopidogrel. Because poor responders are not adequately protected from MACE, it has been proposed that antiplatelet therapy should be tailored to each individual patient, based on the results of platelet function tests. Although this approach is generally considered a desirable evolution of modern medicine, which ideally should be tailored based on individual needs, it is in fact an old remedy (of yet unproven efficacy, in the case of antiplatelet therapy) to the problem of response variability to antithrombotic drugs. Treatment with vitamin K antagonists and with unfractionated heparin has always been tailored to the individual patient, based on laboratory monitoring, because the bioavailability of these drugs is unpredictable and highly variable. However, laboratory monitoring is expensive, increases the workload of health personnel, may be inaccurate, is uncomfortable for patients, and may decrease patients’ adherence to treatment\(^{[1]}\). For the above reasons, treatment with anticoagulant drugs is progressively disposing of laboratory monitoring, thanks to the introduction in the clinical practice of new drugs with very good and predictable bioavailability, such as low molecular weight heparins which do not need laboratory monitoring, and have progressively replaced unfractionated heparin. Therefore, it appears that antiplatelet therapy is heading towards an opposite direction compared to anticoagulant therapy.

Laboratory tests to measure the response to antiplatelet drugs

Many studies used various techniques to measure platelet function ex vivo in order to evaluate the degree of its inhibition by anti-platelet treatment and, in some instances, to predict the risk of atherothrombotic events\(^{[2,3]}\). However, depending on the type of test used, the finding of high, residual platelet reactivity in vitro in patients on aspirin or clopidogrel may not necessarily imply that these patients are resistant to treatment, especially when platelet function is measured by laboratory tests that are not specific for the effect of the anti-platelet drug on its pharmacological target. Therefore, only the use of specific tests that measure the pharmacological effect of the anti-platelet drug will clarify whether their platelet hyper-reactivity is due to insufficient pharmacological effect of the drug or to other causes\(^{[4]}\).

Serum TxB\(_2\) reflects the total capacity of platelets to synthesize TxA\(_2\), of which it is a stable metabolite. Because the contribution of other blood cells to its synthesis is marginal, serum TxB\(_2\) is the most specific test to measure the pharmacological effect of aspirin on platelets\(^{[5]}\). Comparison of different laboratory methods usually showed very weak or no correlation in published studies, indicating that they are sensitive to different parameters. Usually, the number of individuals with residual, significant TxB\(_2\) production on aspirin was extremely low, while the prevalence of individuals with no inhibition of platelet function measured by other, less specific tests tended to be much higher\(^{[6]}\).

ADP-induced platelet aggregation measured by LTA may overestimate the prevalence of poor responders to P2Y\(_{12}\) inhibitors, because ADP induces platelet aggregation by interacting with both its platelet receptors, P2Y\(_1\) and P2Y\(_{12}\). The platelet aggregation-based assay VerifyNow P2Y\(_{12}\) and the flow cytometry-based assay Platelet VASP\(^{[6]}\) (which measures the P2Y\(_{12}\)-dependent inhibition by ADP of phosphorylation of vasodilator-stimulated phosphoprotein, VASP), are more specific assays for measuring the effects of thienopyridines and other drugs inhibiting the platelet P2Y\(_{12}\) receptor\(^{[7]}\).

Interindividual variability in response to aspirin

Studies that measured serum TxB\(_2\) to assess the response to aspirin revealed that the prevalence of poor responders is extremely low\(^{[2,4,5]}\). Lack of compliance is probably the most frequent and plausible cause of insufficient inhibition of COX-1 by aspirin\(^{[1,5]}\). Competition of aspirin with other non-steroidal anti-inflammatory drugs, such as ibuprofen, can prevent aspirin access at Ser529 of COX-1 and, as a consequence, its irreversible acetylation and inactivation of the enzyme\(^{[8]}\).

Impaired inhibition of urinary excretion of Tx metabolites has been associated with increased incidence of MACE, and interpreted as due to insufficient inhibition of platelet COX-1\(^{[9]}\). However, considering that atherothrombosis is an inflammatory disease, a possible, alternative interpretation of these results is that high urinary levels of
Interindividual variability in response to clopidogrel

In contrast to aspirin, studies that used specific tests to measure the pharmacological effect of clopidogrel did show a wide variability of response, with a significant proportion of subjects (about 1/3) who are very poor responders, displaying almost no inhibition of platelet function[8].

Clopidogrel is an inactive prodrug, which, in order to exert its antiplatelet effect, needs to be metabolized to its active metabolite by hepatic cytochrome P450 (CYP) isoenzymes in a two-step process[9]. Several lines of evidence strongly suggest that variability in active metabolite generation is the primary explanation for clopidogrel antiplatelet response variability[9]. Loss of function mutations (e.g., CYP2C19*2) and gain of function mutations (e.g., CYP2C19*17) of CYP isoforms are associated with variable degrees of production of the active metabolite and, hence, of the pharmacodynamic response to the drug[9].

Variable levels of active metabolite generation and/or of pharmacodynamic response to clopidogrel are also associated with: 1) limited intestinal absorption, which is associated with the homozygous 3435C→T mutation of ABCB1; 2) interaction with other drugs, including proton pump inhibitors (PPI), calcium channel blockers and statins, which are metabolized by CYP isoforms; 3) pre-existent variability in platelet response to ADP[10]. Other variables that influence the response to clopidogrel include advanced age, high body mass index and diabetes mellitus, which are associated with decreased response to the drug[10]. Finally, noncompliance is to be considered an obvious and frequent cause of poor response to clopidogrel[10].

Several independent studies demonstrated an association between suboptimal generation of the active metabolite, decreased inhibition of platelet function, presence of enzyme polymorphisms and clinical outcomes[9]. However, no study has yet associated all of these parameters in the same patient population, and some uncertainties still persist.

The association between poor clinical outcomes of patients on treatment with clopidogrel and the presence of loss of function mutations of CYP has been demonstrated by several observational and intervention studies. However, the results of meta-analyses of the data gave conflicting results, with the most recent and comprehensive ones failing to demonstrate an association of CYP genotypes and clinical outcomes in patients treated with clopidogrel[9,11].

Two meta-analyses of observational studies, case-control studies and of post-hoc analyses of randomized clinical trials showed that the risk of MACE was higher in patients on combined treatment with clopidogrel and proton pump inhibitors (PPI), especially omeprazole, compared with patients on clopidogrel not in combined treatment with PPI[9,12]. However, the only randomized, placebo-controlled clinical trial that was designed to test prospectively the interaction between clopidogrel and omeprazole, failed to show that the co-administration of omeprazole and clopidogrel increases the incidence of MACE[13].

In conclusion, although it is plausible and likely that suboptimal pharmacodynamic response to clopidogrel is associated with poor clinical outcomes, there still are contrasting reports in the literature, linking negative interactions with the pharmacodynamics response to the drug and negative interactions with clinical outcomes.

Tailored treatment of patients, based on the results of platelet function tests or of CYP genotyping, has been proposed to solve the problem of clopidogrel resistance[8,14]. This approach cannot be recommended in daily clinical practice yet, because the best laboratory method to monitor the effects of clopidogrel on platelet function still needs to be identified, standardized (for pre-analytical and analytical variables) and validated in the clinical setting[8,14]. Several recent studies demonstrated that the agreement among different laboratory tests to identify poor responders is rather low and that assessment of platelet response to clopidogrel is highly test-specific[8,15], Moreover, loss of function mutations of CYP account for only about 10% of the variability of response to clopidogrel, thus explaining the high degree of inaccuracy of CYP genotyping in predicting the response to clopidogrel[8,14]. In addition, preliminary experiments that evaluated the effects of increasing the dose of clopidogrel in resistant patients gave results that were incompletely satisfactory, because many patients remained “resistant” to clopidogrel, even after repeated administrations of high doses of the drug[15].

Our incompetence on personalized antiplatelet treatment has been recently testified by the negative results of GRAVITAS, the first, large randomized prospective trial testing the efficacy and safety of personalized clopidogrel treatment in patients undergoing PCI[16]. GRAVITAS showed that, in patients with high platelet reactivity on clopidogrel (≥ 230 PRU with the VerifyNow-P2Y12 test, 12-24 h after PCI), high-dose clopidogrel (additional 600 mg followed by 150 mg daily) did not reduce the incidence of MACE, nor did it increase the incidence of bleeding, compared with the standard dose (75 mg daily)[16]. A post-hoc analysis of the results of the GRAVITAS trial showed that the choice of a different cut-off value (PRU ≥ 208), according to the indication of a more recent observational study, would have allowed a more accurate identification of poor responders to clopidogrel and, possibly, the therapeutic success that was missed by the GRAVITAS trial[17]. However, the results of RECLOSE 2-ACS, a recently published large, prospective observational study[18] deny that a more accurate identification of poor responders to clopidogrel would be in itself sufficient to secure the success of treating poor responders with high dose of clopidogrel. Indeed, despite the fact that the laboratory test (platelet aggregation induced by ADP, studied with LTA) could predict the risk of MACE of poor responders, the improvement of the pharmacological response to high doses (up to 300 mg daily maintenance dose) of clopidogrel (or to ticlopidine, 500-1000 mg daily) was not associated with a reduction of the incidence of MACE[18]. The criticisms that have been raised to the GRAVITAS trial, the amendments that have subsequently proposed by its authors, and the therapeutic failure of RECLOSE 2-ACS further emphasize our uncertainties and, as a consequence, the prematurity and incorrectness of tailoring clopidogrel treatment based on laboratory tests in the clinical practice.
Conclusion

When properly studied, the pharmacological response to aspirin is quite consistent among different individuals: therefore, there is no need for monitoring aspirin treatment.

As far as clopidogrel is concerned, the strategies of tailored treatments based on laboratory monitoring that have been used so far failed to improve the clinical outcome of patients with poor responsiveness to the drug. Additional strategies of personalized treatment (serial testing, combined patient genotyping and serial testing, use of the new P2Y$_{12}$ inhibitors instead of high-dose clopidogrel in low responders) might prove effective\(^{(14)}\). However, 1) serial testing with or without genotyping will increase the overall cost of treatment, possibly offsetting the advantage of using the cheaper drug clopidogrel instead of the new, more expensive P2Y$_{12}$ inhibitors; 2) the use of the new P2Y$_{12}$ antagonists prasugrel or ticagrelor instead of clopidogrel would eliminate the problem of hyporesponsiveness, because they effectively inhibit platelet function in the vast majority of patients\(^{(14)}\). Both prasugrel and ticagrelor increase the incidence of bleeding, but it is this mostly to be ascribed to the fact that all patients treated with these drugs display a good inhibition of platelet function: all of them are therefore protected from thrombosis and exposed to the risk of bleeding (as opposed to only about 70% of patients treated with clopidogrel)\(^{(14)}\). Accordingly, it has been clearly demonstrated that patients displaying good response to clopidogrel are at higher risk of bleeding than those who are non-responsive\(^{(14)}\). In other words, if all patients were to respond well to clopidogrel (which is the aim of tailored treatment with clopidogrel) they would have a lower incidence of MACE and a higher incidence of bleeding: exactly like prasugrel- or ticagrelor-treated patients. The rate of bleeding complications is related to the degree of inhibition of platelet function, rather than to the type of drug used to cause it. The use of the new P2Y$_{12}$ inhibitors in all patients, without testing, might prove more effective and cost-effective than personalized treatment: this hypothesis should be tested in controlled studies. While we await the results of additional controlled studies, personalized treatment should not yet be implemented in clinical practice.

References