Aspirin and Clopidogrel
Efficacy, Safety, and the Issue of Drug Resistance

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**Abstract**—Aspirin and the thienopyridines ticlopidine and clopidogrel are antiplatelet agents that display good antithrombotic activity. In the past few years, the concept of aspirin resistance has been largely emphasized in the medical literature, although its definition is still uncertain. I suggest that “aspirin-resistant” should be considered as a description for those individuals in whom aspirin fails to inhibit thromboxane A2 production, irrespective of the results of unspecific tests of platelet function, such as the bleeding time, platelet aggregation, or the PFA-100 system. Less well known than aspirin resistance, but certainly better characterized, is the issue of “clopidogrel resistance,” which is probably mostly caused by inefficient metabolism of the prodrug clopidogrel to its active metabolite. At present, aspirin and clopidogrel resistance should not be looked for in the clinical setting, because there is no definite demonstration of an association with clinical events conditioning cost-effective changes in patient management. (Arterioscler Thromb Vasc Biol. 2004; 24:1980-1987.)

**Key Words:** aspirin resistance ■ clopidogrel resistance ■ antiplatelet agents ■ cardiovascular diseases ■ cerebrovascular diseases
synthesis, improvement of endothelial function, and the well-known anti-inflammatory effects.

The meta-analysis of the Antiplatelet Trialists’ Collaboration (ATC) demonstrated a 25% reduction of vascular death, myocardial infarction (MI), or stroke for antiplatelet therapy (primarily aspirin) versus placebo in patients with acute or previous cardiovascular or cerebrovascular events. More recent analysis of the published literature suggested that the indications for aspirin use should be expanded to primary prevention in populations at high risk, such as those with diabetes, peripheral vascular disease, carotid stenosis, end-stage renal disease, or polycythemia vera.

Of paramount importance are the results of the ISIS-2 trial, which showed that aspirin reduces the mortality from acute MI to an extent that is similar to that of the thrombolytic agent streptokinase. Considering that aspirin is very cheap and extremely safe, this finding could have the greatest impact on a lifelong bleeding disorder.

Long-term therapy with aspirin is associated with a modest increase in the incidence of gastrointestinal bleeding. Although it is generally held that the incidence of gastrointestinal bleeding is dose-related, a recent meta-analysis, which included a substantial number of studies that used low-dose aspirin, found no evidence of lower incidence of gastrointestinal bleeding associated with the use of low-dose aspirin.

It must be noted that despite the clear experimental evidence of its efficacy and safety, aspirin use continues to be less than optimal.

**Thienopyridines**

Ticlopidine and clopidogrel are prodrugs, which need to be metabolized in the liver to active metabolites. These irreversibly inactivate the platelet ADP receptor P2Y12, of the 2 G-protein coupled receptors that are expressed on the platelet membrane (Figure 1), the combined action of which is necessary for a full activation and aggregation response to stimulation by ADP. P2Y12 is negatively coupled to adenylyl cyclase through Gi, mediates a progressive and sustained aggregation not preceded by shape change, and plays an important role in the potentiation of platelet secretion induced by several agonists. Its congenital deficiency results in a lifelong bleeding disorder.

Ticlopidine (250 mg twice daily) is an efficacious antithrombotic agent in patients with claudication, unstable angina, peripheral artery bypass surgery, and cerebrovascular disease.

Clopidogrel (75 mg daily) was compared with 325 mg aspirin in the CAPRIE trial, which enrolled patients at risk for ischemic events because of previous MI, ischemic stroke, or peripheral artery disease. The trial showed an 8.7% relative risk reduction of the major end points (MI, ischemic stroke, and vascular death) in patients treated with clopidogrel compared with patients treated with aspirin. The absolute risk reduction was only 0.9% and the number needed to treat was 115 (95% CI, 58 to 8647), which, given the high cost of the drug, renders its cost-effectiveness unattractive in these patients.

Treatment with ticlopidine is associated with a high incidence of neutropenia (~1%), which is usually reversible on discontinuation of treatment; however, in a few cases, it is irreversible and potentially fatal. Patients must be periodically monitored, especially in the first 3 months of treatment, to detect this harmful complication. Another potentially life-threatening complication of ticlopidine therapy is thrombotic thrombocytopenic purpura. Clopidogrel represents an advance in antplatelet therapy because, compared with ticlopidine, its use is not complicated by neutropenia. It must be noted, however, that thrombotic thrombocytopenic purpura is still a harmful, albeit very rare, complication of clopidogrel treatment.

**Combined Therapy With Aspirin and Thienopyridines**

Theoretically, inhibition of the 2 main amplification pathways of platelet aggregation, the ADP and the arachidonate/ TxA2 pathways, is superior to inhibition of either pathway alone in preventing thrombus formation. As a matter of fact, dual antiplatelet therapy with aspirin plus ticlopidine is superior to aspirin alone, and also to aspirin plus warfarin, in patients undergoing coronary stent implantation. The combination of clopidogrel and aspirin seems to be at least as
The CURE study showed that the addition of clopidogrel to aspirin reduced by 20% the incidence of vascular end points in patients with unstable angina or non-ST segment elevation MI. The PCI-CURE substudy showed also that patients undergoing percutaneous revascularization benefit from dual antiplatelet therapy. Finally, the CREDO trial showed that dual antiplatelet therapy should be continued beyond the usual 30 days because after 1-year treatment, patients in dual therapy experienced a 27% relative risk reduction in death, MI, and stroke compared with patients who were assigned to aspirin alone after the first 30 days of treatment with clopidogrel and aspirin.

Kastrati et al showed that patients with low or intermediate risk who had been treated with aspirin and a 600-mg loading dose of clopidogrel before stent implantation do not have additional benefit from abciximab infusion. Theoretically, abciximab is the antiplatelet agent with highest antithrombotic activity, because it blocks the binding of adhesive proteins to GPIIb/IIIa on activated platelets, which represents the final common and essential pathway for platelet aggregation, thereby inhibiting platelet aggregation irrespective of the type and number of platelet agonists that triggered it. Therefore, the study by Kastrati et al demonstrates that the combined inhibition of the arachidonate/TxA2 and the ADP pathways can achieve an optimal antithrombotic effect.

Unfortunately, combined therapy with thienopyridines and aspirin is associated with an increased risk of hemorrhagic complications, which can require blood transfusion, especially in patients undergoing coronary revascularization.

The Issues of Resistance to Aspirin and Resistance to Clopidogrel

In the past few years, the problem of “aspirin resistance” has been largely emphasized in the medical literature, although its definition and probably even its real existence are still uncertain. More recently, clopidogrel resistance has also been investigated.

The term “resistance” to a drug should be used when a drug is unable to hit its pharmacological target, because of inability to reach it (as a consequence of reduced bioavailability, in vivo inactivation, negative interaction with other substances) or because of alterations of the target.

Aspirin Resistance

Definition

The term “aspirin resistance” has been given different definitions by different researchers, and I think that an effort should be made to propose a universally acceptable definition of this phenomenon. A list of definitions that have been given to “aspirin resistance” follows, with my personal considerations relative to each of them.

Failure of Aspirin to Inhibit Platelet Function In Vivo or In Vitro

Platelet function in vivo has been measured by the bleeding time, whereas platelet function in vitro has, in most instances, been measured by light transmission aggregometry or by global techniques that evaluate primary hemostasis, such as the PFA-100 system or, more rarely, the Utegra Rapid Platelet Function Assay-ASA. All these techniques, albeit to different degrees, are sensitive to several variables. Among these, platelet TxA2 production, which is the pharmacological target of aspirin, is usually of marginal importance, and one should not expect aspirin to inhibit completely platelet functions that are not regulated by TxA2 only.

Most published studies used inadequate techniques to measure the response to aspirin and/or had an inadequate experimental design.

The bleeding time is a highly inaccurate and poorly reproducible technique, which is dependent on several variables, including platelet function, platelet count, plasma factors, red blood cells, and the vessel wall. As a consequence, it displays a low sensitivity to mild abnormalities of primary hemostasis, such as type 1 von Willebrand disease, platelet secretion defects, and drug-induced platelet dysfunction. It is therefore not surprising that aspirin does not prolong the bleeding time of many individuals, because the inhibition of TxA2-dependent platelet function can be easily outweighed by other variables that cannot be affected by aspirin. Therefore, the bleeding time is an improper method to measure platelet inhibition by aspirin.

Light transmission aggregometry measures the increase in light transmission through a platelet suspension that occurs when platelets are aggregated by an agonist. There are many pre-analytical and analytical variables that affect the results of platelet aggregation. Even when all of them are controlled for, the accuracy and reproducibility of the technique are very poor. In addition, the results obtained within one laboratory can barely be compared with those obtained in a different laboratory because of lack of standardization. For instance, the source of the platelet agonists, the scales of the recorder (arithmetic versus logarithmic), and the geometry of the optical system all influence the results of platelet aggregometry. Therefore, any attempt to define universal cutoff values of platelet aggregation to identify nonresponders to antiplatelet therapies would be pointless. In addition to the aforementioned general problems, platelet aggregometry is not ideal antithrombotic agents, that are used to prevent multifactorial diseases, such as those associated with vascular occlusions. Aspirin inhibits only 1 pathway of platelet aggregation. Platelet aggregation is only 1 mechanism regulating thrombus formation; thrombus formation is the most common, but not the only, mechanism leading to vascular occlusion. Vascular occlusion causes clinical events that can be widely different in terms of severity, ranging from asymptomatic in some patients to lethal in others. Given this picture, it would be unreasonable to expect aspirin, or any antithrombotic drug, to prevent clinical events in all patients at risk. Therefore, the definition of “aspirin resistance” that is based on clinical outcomes is certainly unacceptable.
Another platelet agonist that has been used in human citrated PRP, ADP, at 10 μmol/L, induces full platelet aggregation that is largely independent of the aggregation response to ADP (Figure 2). Arachidonic acid, being the precursor of TxA2, is certainly a more suitable platelet agonist than ADP for studying the effects of aspirin. However, the final platelet aggregation induced by arachidonic acid is the sum of the effects of synthesized TxA2 and other agonists secreted by the platelet granules. In addition, 0.5 mg/mL arachidonic acid is a rather high concentration, which may cause some degree of platelet lysis in vitro, causing an increase in light transmission through PRP that cannot be easily distinguished from that caused by platelet aggregation. Another platelet agonist that has been used in studies of aspirin resistance is collagen. Collagen-induced platelet activation is a function of platelet reactivity to collagen, platelet production of TxA2, and platelet responsiveness to secreted ADP. Therefore, also, collagen-induced platelet aggregation is far from being the ideal specific test for measuring aspirin response.

The PFA-100 system could be considered an in vitro bleeding time. It creates an artificial vessel consisting of a sample reservoir, a capillary, and a biologically active membrane with a central aperture, coated with collagen plus ADP or collagen plus epinephrine. The application of a constant negative pressure aspirates the anticoagulated blood of the sample from the reservoir through the capillary (mimicking the resistance of a small artery) and the aperture (mimicking the injured part of the vessel wall). A platelet plug forms that gradually occludes the aperture; as a consequence, the blood flow through the aperture gradually decreases and eventually stops. The time needed to blood flow interruption (“closure time”) is recorded. Compared with the bleeding time, the PFA-100 system is more reproducible and more sensitive to type 1 von Willebrand disease. However, like the bleeding time, it is sensitive to many variables, including platelet function, platelet count, red blood cells, and plasma von Willebrand factor (VWF). Therefore, the effects of inhibition of TxA2, which is associated with mild prolongation of the closure time of the collagen–epinephrine cartridge, can be easily outweighed by other variables that cannot be affected by aspirin. This could easily account for the high percentage of subjects with short closure time despite being on aspirin treatment. Because the PFA-100 system studies platelet function under flow conditions that are characterized by high shear, plasma VWF is a major determinant of closure time. As a matter of fact, Chakroun et al recently showed that high plasma VWF levels are the main determinant of short PFA-100 closure time in patients with cardiovascular disease on aspirin treatment, and Watala et al demonstrated that VWF interaction with GPIb and GPIIb/IIIa is the major determinant of PFA-100 closure time, whereas other platelet receptors and mechanisms leading to platelet aggregation are of minor significance. These findings explain the relatively high prevalence of short PFA-100 closure time in patients on aspirin treatment for cardiovascular or cerebrovascular disease, because these patients tend to have higher than normal levels of plasma VWF. Therefore, because aspirin does not inhibit the major determinants of the PFA-100 closure time, it appears that the PFA-100 system is not an adequate method to measure platelet inhibition by aspirin. A clear demonstration of this comes from the study of Andersen et al, who showed that aspirin treatment abolished TxB2 production to the same extent in patients with short closure time (“aspirin resistant”) and patients with long closure time (“aspirin sensitive”).

The Ultegra Rapid Platelet Function Assay-ASA measures the agglutination of fibrinogen-coated beads by platelets stimulated by an agonist in citrated whole blood. Although the specificity of the test for aspirin inhibition is reported to be 85% by the manufacturer, it is also sensitive to GPIIb/IIIa inhibitors, dipyridamole, clopidogrel, and streptokinase, suggesting that it is far from the ideal specific test for measuring the effect of aspirin on platelets.

Many studies failed to compare the results obtained before aspirin ingestion with those after aspirin. Given the high
Another mechanism of aspirin resistance that should never be laid aside is lack of compliance, which, in a recent study, accounted for the majority of poor aspirin response and was the only significant mediator of poor clinical outcome.

Unproven Aspirin Resistance

The mechanisms responsible for insufficient platelet function inhibition during aspirin therapy should be looked for among the several aforementioned variables that affect the platelet function tests that have been used: increased sensitivity to ADP-induced GPIIb-IIIa activation, increased resistance to collagen, high plasma levels of VWF, GPIIb-IIIa polymorphisms, among others. In addition, the role of a nonenzymatic, oxidation-dependent pathway for the synthesis of the arachidonic acid derivatives isoprostanes, which exhibit potent proaggregatory activity, should also be considered. Factors related to the subject, such as hyperlipidemia, cigarette smoking, and physical or mental stress, could also play a role. Two reports showed that the extent of inhibition of platelet aggregation by aspirin progressively decreased over time in some patients, suggesting that some kind of aspirin tolerance may develop during chronic aspirin treatment.

Clinical Consequences

True Aspirin Resistance

Eikelboom et al showed that suboptimal reduction of urinary 11-dehydro TxB₂ levels during aspirin treatment is associated with heightened risk for future MI and cardiovascular death, indeed suggesting that “true” aspirin resistance may be a clinically relevant phenomenon. Inadequate inhibition by aspirin of TxA₂ biosynthesis can be observed in patients on treatment with ibuprofen, because of competition of the 2 drugs at the COX-1 level. Observational studies and post hoc analysis suggested that ibuprofen blunts the cardioprotective effect of aspirin, although the question is still controversial.

Unproven Aspirin Resistance

An association between suboptimal platelet function inhibition during aspirin treatment and heightened incidence of cardiovascular or cerebrovascular events has been described. These interesting findings, if confirmed in larger studies, could bear important clinical implications, because they suggest that monitoring platelet function during antiplatelet therapy can be useful to predict the risk of treatment failures. However, the phenomenon that they describe should not be termed “aspirin resistance,” because it is determined to a large extent by variables that cannot be inhibited by aspirin.

In my opinion, the available evidence of the predictive value for cardiovascular events of laboratory tests evaluating either “true” or “unproven” aspirin resistance is insufficient to recommend laboratory monitoring of patients on aspirin treatment in the clinical setting.

Clopidogrel Resistance

Less well known than aspirin resistance, but certainly better characterized, is “clopidogrel resistance.”
**Definition**

Correctly, the term has never been used to refer to treatment failures, despite the fact the clopidogrel is only marginally superior to aspirin in preventing cardiovascular events.26

The extent of the platelet aggregation response in vitro to ADP has been used to define “clopidogrel resistance” in the large majority of studies that have been published so far. Needless to say, the aforementioned general pitfalls of in vitro platelet aggregation apply not only to studies of “aspirin resistance” but also to those of “clopidogrel resistance.” In addition, although ADP is the most appropriate aggregating agent in this context, because clopidogrel antagonizes the ADP receptor P2Y12, it must be noted that platelets express also a second ADP receptor, P2Y1, which causes the initial wave of ADP-induced platelet aggregation (Figure 1).2 Because the extent of residual, P2Y1-dependent platelet aggregation induced by ADP varies widely among patients with congenital P2Y12 deficiency or normal subjects in whom P2Y12 function had been completely blocked in vitro by saturating concentrations of specific antagonists, ADP-induced platelet aggregation may not be the most suitable test to measure the individual response to clopidogrel. A better and more specific test would be measurement of the extent of ADP-induced inhibition of adenyl cyclase, which is uniquely mediated by P2Y12. This could be accomplished by measuring the inhibition by ADP of PG-induced platelet cAMP increase or phosphorylation of vasodilator-stimulated phosphoprotein.84

**Mechanisms**

Clopidogrel (like ticlopidine) is a prodrug, which needs to be metabolized by the liver to an active metabolite with antiaggregating activity (Figure 1).23 Therefore, its pharmacological effect can be detected only some time after its first administration and, more importantly, the plasma levels of the active metabolite vary widely among subjects. Consequently, the degree of inhibition of platelet aggregation induced by ADP varies widely among subjects. In published studies, ~50% of the patients were either clopidogrel nonresponders or low responders.85 Interindividual variability in platelet inhibition by clopidogrel correlated well with the metabolic activity of the hepatic cytochrome P450, which activates the prodrug to its active metabolite.86 Whether polymorphisms of the clopidogrel target, P2Y12, play additional roles in modulating the individual response is presently unknown.87 Interference with clopidogrel metabolism by other drugs that are frequently given to patients with atherosclerosis, such as atorvastatin,86,88 can increase the number of patients who are resistant to clopidogrel, although this is still a controversial issue.89,90

**Clinical Consequences**

A recent study of 60 patients undergoing coronary angioplasty confirmed the high interindividual variability of platelet inhibition by clopidogrel and showed that patients with clopidogrel resistance (mean ADP-induced platelet aggregation on day 6 of treatment=103±28% of baseline) are at increased risk for recurrent cardiovascular events.91

**Conclusions**

Aspirin and the thienopyridines ticlopidine and clopidogrel are antiplatelet agents that display good antithrombotic activ-

**Recommendations for Studying Aspirin and Clopidogrel Resistance**

1. Rule out patient noncompliance
2. Measure the function of specific targets of the antiplatelet drugs:
   a. COX-1 for aspirin
      (laboratory measurement: levels of serum TxB₂ or urinary TxB₂ metabolites)
   b. P2Y₁₂ for clopidogrel
      (laboratory measurement: inhibition by ADP of prostaglandin-induced increase in platelet cyclic AMP or phosphorylation of VASP)
3. Measure the “response” to the antiplatelet drugs: baseline vs after treatment

At present, aspirin and clopidogrel resistance should be studied for investigational purposes only. They should not be looked for in the clinical setting, because no definite demonstration of an association with clinical events conditioning cost-effective changes in patient management is available yet.

**References**


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