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

Aspirin
Aspirin irreversibly inhibits COX-1 by acetylation of a serine residue at position 530, thereby preventing the conversion of arachidonate to the unstable prostaglandin (PG) intermediate PGH2, which is converted to TxA2, a potent vasoconstrictor and platelet agonist (Figure 1). A single dose of 160 mg completely abolishes the platelet TxA2 production (measured as its stable analogue TxB2). The same effect can be progressively achieved with the chronic administration of daily doses of 30 to 50 mg.1 COX has 2 isoforms with different tissue distribution and susceptibility to inhibition by NSAID. COX-2 in not inhibited by therapeutic doses of aspirin and, under physiological conditions, is present in a small fraction of platelets,3-5 but the number of COX-2—expressing platelets may increase in conditions of high platelet regeneration.5

Because aspirin acetylates COX-1 in all tissues, including endothelial cells, where the enzyme converts arachidonic acid into the vasodilator and natural platelet antagonist prostacyclin, for several years there has been the concern that the potential antithrombotic effect of aspirin could be blunted, or even overcome, by the theoretical prothrombotic effect associated with the parallel inhibition of prostacyclin.6 The search for low doses of aspirin that could completely inhibit platelet COX-1 while sparing endothelial cell COX-1 has been intensive for several years. However, based on the positive results of clinical trials, which mostly used high doses of aspirin, it can be safely concluded that doses of aspirin that inhibit both platelet and endothelial cell COX-1 are antithrombotic, indicating that the protective effect of platelet COX-1 inhibition outweighs the theoretical prothrombotic effect of endothelial COX-1 inhibition. It must be noted that high doses of aspirin might have antithrombotic effects that are independent of platelet COX-1 inhibition, including increased fibrilolytic activity,7 depression of prothrombin...
synthesis, improvement of endothelial function, and the well-known antiinflammatory 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 acute MI-associated mortality worldwide than any other, albeit very important, achievement in this field.

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, one 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 antiplatelet 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
Vascular Occlusion Failure of Aspirin to Prevent Clinical Events Associated to Vascular Occlusion

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 Prevent Clinical Events Associated to Vascular Occlusion
This phenomenon has been called “clinical aspirin resistance,” but it should be termed “treatment failure.” It can be observed with any kind of treatment and is expected to be particularly frequent for drugs, like aspirin and all other 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.

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.

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 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...
Human Citrated PRP

Before aspirin

After aspirin

Light transmission

ATP release

Figure 2. Tracings of platelet aggregation (upper) and ATP secretion (lower) recorded simultaneously with a luminaggregometer (Chronolog). ADP, at the indicated concentrations (2, 4, and 10 μmol/L), was added to samples of citrated PRP obtained from a healthy volunteer before (left tracings) and 2 hours after the oral administration of 100 mg aspirin (right tracings). The results of the experiment show that the aspirin-inhibitable component of ADP-induced human platelet aggregation in citrated PRP decreases by increasing the concentration of ADP. At 10 μmol/L, ADP induces platelet aggregation that is practically unaffected by aspirin treatment. In contrast, aspirin completely abolished the TXA2-dependent platelet secretion at all the ADP concentrations tested. Therefore, platelet aggregation induced by 10 μmol/L ADP is not the ideal test to measure platelet response to aspirin treatment (see text).

for testing platelet sensitivity to aspirin because, depending on the agonist used and its concentration, the aggregation response is only partially and variably modulated by TXA2. For instance, Gum et al defined aspirin resistance as a mean aggregation of ≥70% with 10 μmol/L ADP and ≥20% with 0.5 mg/mL arachidonic acid.42,43 Although aggregation-dependent TXA2 production can potentiate ADP-induced platelet aggregation in citrated PRP,44,45 ADP, at 10 μmol/L, induces full platelet aggregation that is largely independent of TXA2 production. It is only at the intermediate concentrations of ∼2 to 4 μmol/L that TXA2 production causes amplification 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.46 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.47-50 Collagen-induced platelet activation is a function of platelet reactivity to collagen,47,51,52 platelet production of TXA2, and platelet responsiveness to secreted ADP.53 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.54 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.55,56 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,41 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.52,45,57-62 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,61 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.63 These findings explain the relatively high prevalence of short PFA-100 closure time in patients on aspirin treatment for cardiovascular or cerebrovascular disease,52,45,57-62 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”).57

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,64,65 it is also sensitive to GPIIb/IIIa inhibitors, dipyridamole, clopidogrel, and streptokinase.65 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...
interindividual variability of platelet function tests, this could lead to inaccurate and somewhat paradoxical conclusions. For instance, studies with PFA-100 usually defined those subjects whose collagen–epinephrine closure time was lower than the upper limit of the normal range (the normal range is between ~80 seconds and 180 seconds) as aspirin-resistant. Let us consider 2 hypothetical subjects. Subject 1 has a baseline closure time of 80 seconds, which increases to 179 seconds during aspirin treatment. Subject 2 has a baseline closure time of 180 seconds, which remains unmodified during aspirin treatment. Paradoxically, a study that measures closure time during aspirin treatment only would classify subject 1 as “aspirin-resistant” and subject 2 as an “aspirin responder.” To avoid this kind of paradoxical result, studies of platelet function should be performed both before and after aspirin intake.

Failure of Aspirin to Inhibit \( \text{TxA}_2 \) Production

Lacking a reproducible and highly sensitive and specific method to study \( \text{TxA}_2 \)-dependent platelet function, the pharmacological response to aspirin treatment should be assessed by measuring the degree of inhibition of \( \text{TxA}_2 \) production. This could be performed by measuring either serum \( \text{TxB}_2 \) or the urinary excretion of \( \text{TxB}_2 \) metabolites. Therefore, based on the available techniques, the only acceptable definition of aspirin resistance should rely on the demonstration of an insufficient inhibition of \( \text{TxA}_2 \) production.

For the sake of clarity, in the remaining part of this review, I refer to failure of aspirin to inhibit \( \text{TxA}_2 \) production with the term “true” aspirin resistance, and to failure of aspirin to inhibit platelet function “in vivo” or “in vitro” (without demonstration of inadequate inhibition of \( \text{TxA}_2 \) production) with the term “unproven” aspirin resistance.

Mechanisms

True Aspirin Resistance

The following potential mechanisms could be considered responsible for “true” aspirin resistance: (1) decreased bioavailability of aspirin; (2) competition of aspirin with other NSAIDs (such as ibuprofen) preventing aspirin access at Ser530 of COX-1; (3) accelerated platelet turnover, introducing newly formed, nonaspirinated platelets into blood stream; (4) transcellular formation of \( \text{TxA}_2 \) by aspirinated platelets from PGH\(_3\) released by other blood cells or vascular cells; (5) \( \text{TxA}_2 \) production by the aspirin-insensitive COX-2 in newly formed platelets or other cells; and (6) (theoretical) presence of variant COX-1 that is less responsive to aspirin inhibition. In patients undergoing coronary artery bypass surgery, Zimmerman et al showed that aspirin inhibition of \( \text{TxA}_2 \) biosynthesis both in vitro and ex vivo is compromised within several days after surgery. Despite the fact that immunoreactive COX-2 in platelets was increased 16-fold, it appeared not to be responsible for aspirin resistance, because a specific COX-2 inhibitor did not affect \( \text{TxA}_2 \) production. In contrast, Kearney et al showed that coronary angioplasty is associated with increased \( \text{TxA}_2 \) formation, which is completely abolished by aspirin.

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 responsiveness 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. This issue is controversial, because another study showed that 100 patients on chronic aspirin treatment had consistently reduced platelet aggregation over time.

Clinical Consequences

True Aspirin Resistance

Eikelboom et al showed that suboptimal reduction of urinary 11-dehydro \( \text{TxB}_2 \) 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 \( \text{TxA}_2 \) 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 In addition, 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 adenylyl 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).25 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±8% 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 there is no definite demonstration of an association with clinical events conditioning cost-effective changes in patient management. For investigational purposes, aspirin and clopidogrel resistance should be evaluated in compliant patients by studying the specific target of each drug. Measurements should be performed both before and after drug administration (Table). 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.

**References**


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