Aspirin Resistance

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Editorial

Roberto Ferrari, MD, PhD
David J. Hearse, BSc, PhD, DSc

“MASTERS OF OUR TRADE”

In the past when resources and equipment were scarce, both artisans and doctors used to rely on a combination of passion and experience to do their jobs. You might think it strange to question the precious gifts of passion and experience that enable a humble apprentice to grow into a skilled master craftsman. However, the ambitions of our esteemed artisan, no matter how great his passion, will always be constrained if he does not possess the right tools to become a master. Like our friend the artisan, we too as physicians at all levels should seize every opportunity to use all the “tools” available, such as techniques, information, and studies, to improve our effectiveness as doctors.

One of our former limitations was a lack of experience. Historically speaking, a cardiologist was only able to gain experience by working on a one to one basis with patients. Obviously many patients were needed to enhance one’s knowledge. Globalization has hopefully enlarged our small-town expertise with the advent of clinical trials on an international scale, with the recruitment of thousands of patients.

As a result, our medical advances can now successfully claim to be evidenced-based. With this new “tool,” pharmacological treatments such as aspirin and clopidogrel have been developed and tested in clinical trials. We know these drugs are effective, but we also know that they are not equally effective in all patients and sometimes cause side effects. Reviewing the current status of antiplatelet treatment analysis, the focus of this issue, requires that we use all the available tools to improve the care we give. In other words, to absorb and evaluate newly gained evidence, while at the same time calling on our own personal experience to tailor our patients’ treatment on an individual basis.

The author of our lead article, Professor Carlo Patrono from the Istituto di Farmacologia at the University Cattolica del S. Cuore in Rome, comments on the clear contrast between the ideal and real world in patient treatment. Real life means that instead of an ideal standard pharmacological reaction, we are faced with the problem of dealing with an immense variety of drug resistance to antiplatelet therapy. The dilemma lies...
in determining which tests are the most suitable for diagnosing the cause of this resistance, which if properly chosen result in the selection of more appropriate treatments for individual patients.

John W. Eikelboom from the McMaster University in Canada and Graeme J. Hankey from the Royal Perth Hospital in Australia shed light on how we still have a long way to go in diagnosing antiplatelet drug resistance using point-of-care assays at the patients’ bedside. Dominick J. Angiolillo, from the University of Florida College of Medicine in Jacksonville, then gives us his point of view on how this phenomenon of antiplatelet reactivity affects high-risk patients, and Marco Valgimigli and Antonella Scalone from the University Hospital of Ferrara in Italy illustrate how some proven therapeutic strategies can be applied to overcome poor antiplatelet responsiveness.

Although progress has been great, we still lack complete and comprehensive guidelines for dealing with poor responsiveness to antiplatelet therapy. Many clinical studies are currently underway and will, we hope, eventually provide us with the clinical evidence that will modify our strategies.

The career of today’s physician is continuously evolving as patients’ needs require him/her to keep abreast of all clinical challenges. Likewise, the experienced artisan finds himself in the same position, learning different, more innovative techniques and work practices to keep up with changing demands. But this must be done without forgetting his origins and his natural talent. In today’s increasingly complex world, the competent artisan must take care to avoid losing touch. The same can be said for today’s physician in what sometimes seems to be a maelstrom of global confusion.

Both have the opportunity to grow and improve their practices by properly using the right tools to make their passion and experience go further than they could ever have imagined possible. And it is this search for betterment, not of being the best, that makes them all “Masters of their Trade.”
Interindividual variability in response to aspirin and P2Y\textsubscript{12} blockers in patients at high cardiovascular risk—the state of evidence

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In an ideal world, 100 patients given the same dose of the same drug would display the same pharmacological response, implying identical pharmacokinetics (what the body does to the drug) and pharmacodynamics (what the drug does to the body). The real world, however, is one of interindividual pharmacokinetic and pharmacodynamic variability. How this determines the response to aspirin and platelet adenosine diphosphate receptor (P2Y\textsubscript{12}) blockers is the subject of this review, designed to help cardiologists critically assess the overwhelming literature on “resistance” to these antplatelet drugs and gauge the utility and limitations of current biochemical, functional, and genetic tests of individual drug response. The term “resistance” is uninformative of the mechanism(s) behind interindividual variability in response and potentially misleading, implying we have a standardized method of measurement directly reflecting clinical efficacy that can dictate changes in antplatelet therapy. In fact, the relationship between the functional indices of platelet capacity measurable ex vivo and the occurrence of platelet activation and inhibition in vivo is far from established. We therefore suggest abandoning the term “resistance” if we are to advance our understanding of the complex determinants of interindividual variability in response to aspirin and P2Y\textsubscript{12} blockers discussed here and elsewhere in this issue.

In an ideal world, one hundred patients given the same dose of a certain drug would be expected to display the same pharmacological response, be it biochemical (eg, a reduction in blood cholesterol), functional (eg, a decrease in blood pressure [BP]), or clinical (eg, an attenuation of pain) in nature. This would imply that all patients absorb the drug, transport it to the site(s) of action, and metabolize and eliminate it in the same fashion, both qualitatively and quantitatively. In one word, they would share identical pharmacokinetics (what the body does to the drug). This would also imply that, when exposed to a given plasma concentration of the drug, the pharmacological target (eg, a platelet enzyme or receptor) would respond equally in all patients. In other words, they would share identical pharmacodynamics (what the drug does to the body).

In the real world, it is common experience that these one hundred patients would display variable pharmacological responses, and this fact is known as interindividual variability in drug response, which reflects both pharmacokinetic (PK) and pharmacodynamic (PD) variability. Terms like hypo- and hyperresponsiveness are often used to designate lower-than- and higher-than-expected responses, respectively. One extreme of this variability is represented by drug resistance, a phenomenon that has been well characterized in the field of chemotherapy. For example, some bacterial strains may become resistant to a given antibiotic because they undergo genetic mutations associated with structural changes in the drug target or with the capacity to synthesize novel enzymes degrading the drug. This phenomenon (in many different nuances) has been described with a variety of antibiotic, antiviral, and anticancer drugs, and two practical corollaries of drug resistance have evolved as a natural consequence: (i) drug resistance can be diagnosed with an appropriate in vitro test; and (ii) once diagnosed, this will lead to a rational change in therapy.
Although no comparable phenomena have been described in the field of cardiovascular drugs, the term “aspirin resistance” was introduced some 20 years ago to designate less-than-expected inhibition of platelet aggregation in response to standard doses of the drug.\(^1\)\(^2\) Similarly, the concept of “clopidogrel resistance” has emerged during the past 10 years based on similar functional measurements.\(^2\) Moreover, because these abnormal measurements of platelet aggregation in aspirin- and/or clopidogrel-treated patients have been associated with enhanced risk of atherothrombotic events in a large series of small observational studies and their meta-analyses,\(^3\)\(^4\) the same practical corollaries of drug resistance noted above have evolved as a natural consequence of this clinical paradigm. However, in contrast to antibiotic resistance, there is no agreement on the most appropriate test(s) to diagnose “resistance” to aspirin or clopidogrel. Moreover, there is no randomized clinical evidence that changing antiplatelet therapy in light of such a diagnosis is a more effective strategy than maintaining standard therapy.

In this lead article, we intend to review the PK and PD determinants of the interindividual variability in response to aspirin and P2Y\(_{12}\) blockers, with the aim of enabling the practising cardiologist to critically assess the overwhelming literature on “resistance” to these antiplatelet drugs as well as to evaluate the potential utility and limitations of currently available biochemical, functional, and genetic tests to evaluate drug response in individual patients.

### DRUG ABSORPTION AND BIOAVAILABILITY

Absorption is the movement of a drug from its site of administration into the central compartment and the extent to which this occurs. Both aspirin and P2Y\(_{12}\) blockers are rapidly and extensively absorbed. However, a more clinically relevant PK parameter is represented by bioavailability, ie, the fraction of the administered dose of the drug that ultimately reaches the systemic circulation from which the drug has access to its site of action. In the case of aspirin, oral bioavailability of intact acetylsalicylic acid is approximately 50% because a fraction of the administered and absorbed dose of the drug is metabolized, ie, deacetylated, by the liver before it enters the systemic circulation (the first-pass effect). Moreover, deactivation of acetylsalicylic acid by hydrolysis to salicylate may also occur in the gut and blood. Aspirin is rapidly absorbed from the stomach, where the pH is low and hydrolysis is minimal. However, enteric-coated formulations of aspirin, now widely used in cardiovascular prevention, release the drug into the upper small intestine, where slower absorption and a more alkaline milieu may facilitate hydrolysis to salicylate, and thus bioavailability of acetylsalicylic acid from these formulations may be reduced.\(^5\) It is important to remember that the mechanism by which aspirin inhibits platelet function requires intact acetylsalicylic acid in order to permanently inactivate a key enzyme (cyclooxygenase [COX]-1) of platelet arachidonic acid metabolism.\(^6\) Although acetylation of platelet COX-1 by aspirin is largely presystemic, ie, occurring in portal blood during the first encounter of the intact drug with circulating platelets, adequate systemic bioavailability of acetylsalicylic acid is required for the acetylation of another important

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**SELECTED ABBREVIATIONS AND ACRONYMS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTIVE A</td>
<td>Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events A</td>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<td>BP</td>
<td>blood pressure</td>
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<td>Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events</td>
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<td>Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance</td>
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<td>COX-1</td>
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<td>CURE</td>
<td>Clopidogrel in Unstable angina to prevent Recurrent Events</td>
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<td>Clopidogrel optimal loading dose Usage to Reduce Recurrent EveNTs-Organization to Assess Strategies in Ischemic Syndromes</td>
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<td>HOPE</td>
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<td>LDL</td>
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<tr>
<td>NSAID</td>
<td>nonsteroidal anti-inflammatory drug</td>
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<td>pharmacodynamic</td>
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<tr>
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<td>PLATelet inhibition and patient Outcomes</td>
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<td>TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibitionN with prasugrel-Thrombolysis In Myocardial Infarction</td>
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<tr>
<td>TXA(_2)</td>
<td>thromboxane A(_2)</td>
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<td>TXB(_2)</td>
<td>thromboxane B(_2)</td>
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<tr>
<td>VASP</td>
<td>vasodilator-stimulated phosphoprotein</td>
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drug target, COX-1 in bone marrow megakaryocytes, a key determinant of the long-lasting antiplatelet effect of low-dose aspirin \(^5,6\) (see below).

Thienopyridines (ticlopidine, clopidogrel, and prasugrel) are prodrugs that require conversion to an active thiol metabolite by the liver cytochrome P450 (CYP) enzyme system in order to permanently inactivate another critical platelet protein, the adenosine diphosphate (ADP) receptor P2Y12. \(^5,7\) Although these drugs are extensively and rapidly absorbed after oral dosing, there are important differences in their metabolic pathways \((\text{Figure 1})\) and systemic bioavailability of the respective active metabolites. \(^7\) Ticlopidine is metabolized by five main pathways resulting in a vast array of mostly inactive metabolites. \(^7\) Of these, one platelet-active metabolite has been identified. However, no detailed studies have been conducted to characterize the CYP isoforms involved in metabolic activation of ticlopidine, and the functional consequences of CYP polymorphisms and drug interactions with CYP-inhibiting drugs are largely unknown.

Clopidogrel is largely inactivated by esterases present in the intestine, liver, and blood. The variable residual amount of the prodrug presented to the liver is metabolized through two CYP-catalyzed steps to a thiol-containing active metabolite responsible for the antiplatelet effect of the drug. CYP3A4/5, CYP2C9, and CYP2C19 appear to be critically involved in active metabolite formation. \(^7\) Loss-of-function genetic variants of these CYP isoforms (eg, CYP2C19*2) are associated with reduced plasma concentrations of clopidogrel’s active metabolite and lower-than-expected inhibition of ADP-induced platelet aggregation. \(^7\) Prasugrel is characterized by a more efficient PK profile than clopidogrel. The same esterases that degrade clopidogrel to inactive metabolites are responsible for the first step of prasugrel’s metabolic activation \((\text{Figure 1})\). The resulting prasugrel thiolactone is converted to the platelet-active metabolite through a single CYP-catalyzed step. Moreover, prasugrel thiolactone is a promiscuous substrate of several CYP isozymes, and its metabolic activation does not appear to be affected by reduced-function CYP polymorphisms. \(^7\)
Three direct and reversible P2Y12 antagonists, cangrelor (an intravenous agent), ticagrelor (an oral agent), and elinogrel (available both as an intravenous and oral agent) are associated with rapid onset and offset of platelet inhibition? Unlike the thienopyridines, they do not require metabolic activation by the liver. Ticagrelor is rapidly absorbed and undergoes enzymatic transformation to at least one active metabolite. Peak plasma concentrations of ticagrelor and maximum platelet inhibition are achieved 1 to 3 hours after dosing. The plasma half-life of ticagrelor is 6 to 13 hours, which dictates a twice daily regimen of administration.7

Thus, in summary, aspirin, ticlopidine, clopidogrel, and prasugrel are characterized by individual PK features that may explain the delivery of variable amounts of the active moiety of the drug to its site(s) of action in different individuals, and therefore provide a PK basis for interindividual variability in pharmacological response. Inadequate bioavailability of intact acetylsalicylic acid or thiol-containing active metabolites of thienopyridines, for galenic or genetic reasons, may be sufficient to explain a reduced antiplatelet effect in some individuals without the need for invoking “resistance” of the drug target. This is particularly true for clopidogrel because its PKs are both uneconomical (most of the administered prodrug is wasted) and risky (its metabolic activation is critically dependent on a single CYP).

**DRUG-TARGET INTERACTION**

Both aspirin and the thienopyridines work through “hit-and-run” PDs, ie, they induce an irreversible modification of their anucleate platelet target, COX-1 and P2Y12 respectively, which explains the long (days) duration of their antiplatelet effects despite the short (minutes) half-life of their active moieties. Permanent inactivation of the platelet target probably represents the most important ingredient of this unique PD mechanism; however, the fact that following withdrawal of aspirin or clopidogrel therapy no apparent recovery of thromboxane A2 (TXA2)- or ADP-dependent platelet function is detectable for about 24 to 48 hours can be interpreted to reflect inactivation of COX-1 and P2Y12 in the platelet progenitors, ie, bone-marrow megakaryocytes.5 While abnormal megakaryopoiesis, as exemplified by the extreme phenotype of essential thrombocythemia, is associated with less-than-expected inhibition of platelet COX-1 by aspirin (a PD interaction).18 Both the acetylation of a strategically located serine residue (Ser-529) within the COX-1 channel by aspirin11 and the formation of a disulfide bridge between the thiol on the active metabolite of clopidogrel and a free cysteine residue in the first extracellular loop of the P2Y12 receptor12 represent molecular processes that could be influenced by genetic variants of the drug target. However, there is no evidence that such a mechanism may account for the frequent occurrence of less-than-expected inhibition of platelet function by either drug. Recent in vitro studies indicate that acetylation of both COX-1 and COX-2 by aspirin is regulated by the peroxidase activity of these enzymes.13 Acetylation occurs most efficiently when the peroxidase activity is low, and acetylation is antagonized by hydroperoxides that are substrates for the peroxidase.13 The functional relevance of these findings is currently being investigated in clinical settings characterized by enhanced lipid peroxidation, such as diabetes mellitus and visceral obesity.

The transient expression of COX-2 in newly formed platelets in disease states characterized by enhanced platelet turnover can contribute, at least in part, to low-dose aspirin-insensitive TXA2 biosynthesis, as demonstrated by the study of patients with essential thrombocythemia.10 Extraplatelet sources of TXA2 (eg, monocytes/macrophages) may also contribute to aspirin-insensitive TXA2 biosynthesis in some patients with acute coronary syndromes15 and acute ischemic stroke.16 However, the failure of a potent TXA2 receptor (TP) antagonist, terutroban, to display superiority vs low-dose aspirin in a large randomized trial of patients with recent cerebrovascular ischemia17 does not lend support to the frequent occurrence of this phenomenon and/or its clinical relevance.

Concomitant administration of a traditional nonsteroidal anti-inflammatory drug (NSAID), such as ibuprofen, may interfere with the irreversible inactivation of platelet COX-1 by aspirin (a PD interaction).18 This is due to competition for a common docking site within the COX-1 channel (arginine-120), to which aspirin has to anchor in order to selectively acetylate Ser-529.11 This interaction has also been reported between naproxen and low-dose aspirin,19 but does not occur with rofecoxib,18 celecoxib,20 or diclofenac,18 ie, drugs endowed with moderate to high COX-2 selectivity.21
Although the clinical consequences of the interaction between aspirin and certain traditional NSAIDs are uncertain, its occurrence in elderly patients with concomitant cardiovascular and osteoarticular diseases may account for a less-than-complete suppression of platelet COX-1 activity in this setting.

A thienopyridine that inactivates the ADP P2Y12 receptor would be expected to suppress ADP-induced platelet aggregation quite profoundly, as suggested by rare mutations in the P2Y12 gene that are associated with a congenital bleeding disorder and abnormality in platelet response to ADP. The fact that ADP-induced platelet aggregation is not inhibited by more than 50% following a loading dose of clopidogrel 600 mg could be interpreted as reflecting “resistance” of the drug target to the active metabolite of clopidogrel. However, when tested in vitro, the active metabolites of clopidogrel and prasugrel had comparable levels of platelet inhibition, ie, they displayed equal potency. As depicted in Figure 2, approximately 1 µM of either metabolite is required in order to achieve approximately 90% suppression of ADP-induced platelet aggregation. However, while a 1 µM concentration of the active metabolite is readily achieved following a loading dose of 60 mg prasugrel, this threshold concentration of the active metabolite is not achievable even following a 5- to 10-fold larger loading dose of clopidogrel (Figure 2) because of its inefficient PKs noted above. Therefore, despite similar PD behavior in vitro, an approximately 10-fold greater exposure to the active metabolite of prasugrel compared to clopidogrel provides an explanation for the faster, greater, and more consistent inhibition of platelet aggregation observed with prasugrel.

Detailed molecular studies of ticagrelor have demonstrated that this selective P2Y12 inhibitor displays apparent noncompetitive antagonism towards ADP-induced receptor activation, suggesting the existence of more than one ligand-binding site on P2Y12. Thus, in summary, there seems to be relatively limited room for PD variability in response to aspirin or thienopyridines, with the notable exception of the PD interaction noted above. Concomitant treatment with some readily available over-the-counter (OTC) NSAIDs may limit the cardioprotective effects of low-dose aspirin (as recently outlined by the Food and Drug Administration [FDA] in a statement for patients and health-care professionals) and contribute, together with nonadherence, to many of the reports of so-called aspirin “resistance.”

**Figure 2. In vitro pharmacodynamics and in vivo pharmacokinetics of active metabolites of clopidogrel and prasugrel.**

**Panel A:** illustrates the effects of active metabolites (AMs) of prasugrel and clopidogrel on human platelet aggregation induced by ADP (10 µM), while **panel B** depicts plasma active metabolite concentrations following the administration of loading doses of prasugrel and clopidogrel. The broken line corresponds to a 1 µM concentration of the active metabolite.

hypertensive drug therapy. Because the latter are rou-
inely evaluated and adjusted according to the individ-
ual low-density–lipoprotein (LDL)-cholesterol and BP
response, respectively, the argument goes that each
patient receiving antiplatelet therapy should be moni-
tored by platelet function testing. However, the analogy
is only apparent if we consider what is being measured.
Both the level of LDL-cholesterol and BP represent in
vivo indexes of lipid and vascular homeostasis, respec-
tively, that can be measured with standardized meth-
ods to monitor drug therapy in the individual patient;
both have been measured in large cohorts of patients
entering long-term observational studies, and their
baseline levels have been related linearly to the occur-
rence of major vascular events and mortality; more-
over, a given reduction in their usual levels achieved
with lipid-lowering or BP-lowering drugs has been
demonstrated to produce a reduction in the rate of ma-
jor vascular events largely consistent with the decrease
predicted by their epidemiological association.28,29

If we now consider the various biochemical (eg, serum
thromboxane B2 [TXB2], vasodilator-stimulated phos-
phoprotein [VASP] phosphorylation) and functional
(eg, agonist-induced platelet aggregation) parameters
that have been used to monitor antiplatelet therapy,
these represent ex vivo indexes of platelet capacity for
performing various activities (eg, releasing free arachi-
donic acid from membrane phospholipids and trans-
forming it to TXA2, releasing their granule content,
expressing surface antigens, undergoing reversible or
irreversible aggregation) in a test tube, under complete-
ly artificial conditions, in response to endogenously
generated (eg, thrombin formed during whole blood
clotting) or exogenously added (eg, variable concen-
trations of arachidonate, ADP, or collagen) agonists,
which gives rise to measurable immunological or elec-
trical signals.30 How these ex vivo capacity indexes re-
late to the occurrence of platelet activation and inhi-
bition in vivo is largely unknown, except for indexes
of platelet TXA2 production that can be measured both
ex vivo and in vivo.8

Moreover, no large longitudinal studies have exam-
ined the relationship between variable levels of these
ex vivo platelet assays and the occurrence of major vas-
cular events during follow-up. In the absence of such
critical information, dose selection in antiplatelet drug
development has been largely empirical, with the no-
table exception of aspirin following clarification of its
molecular mechanism of action and design of a mech-
anism-based biochemical end point, ie, serum TXB2,
for dose-finding studies.30 In the case of clopidogrel,
the selection of 75 mg once daily for the phase 3 piv-
otal trial was based on very limited phase 2 studies
designed to reproduce the same extent of inhibition,
This raises two important questions: is there a threshold of drug target inactivation for full suppression of TXA2- or ADP-dependent platelet function? Moreover, is such a complete PD response achievable and desirable? In the case of aspirin, the answers to these questions are straightforward: greater than 97% inhibition of platelet COX-1 activity is required for maximal suppression of arachidonate-induced platelet aggregation; this PD response is achievable, with daily doses as low as 30 mg producing a ceiling effect on platelet biochemistry and function. These considerations guided the successful development of low-dose aspirin as an antithrombotic agent. However, the recent findings of the clinical development of prasugrel would suggest that a procoagulant response detected by annexing V binding, microparticle formation, and P-selective expression. Interestingly, several assays that are commonly used to monitor platelet function differed in their sensitivity to varying levels of P2Y12 blockade, and 80% or greater receptor blockade was required for consistently strong inhibition of several aspects of platelet function. At least 1µM R-138727 was required to achieve this level of P2Y12 blockade, an active metabolite concentration achievable following a 60 mg loading dose of prasugrel, but not achievable after a 5- to 10-fold higher loading dose of clopidogrel (Figure 2).

In light of these recent findings, one should ask the next ensuing question: is there any justification for selecting a particular threshold of functional responses to ADP for classifying patients as “nonresponders” or “resistant” to clopidogrel? The linearity of the relationship between the degree of P2Y12 blockade and inhibition of P2Y12-mediated platelet function would suggest treating these functional measurements as continuous variables, and does not provide any obvious justification for the common practice of dichotomizing functional responses (“responder” vs “nonresponder”) on the basis of an arbitrary (and variable among studies) threshold. Moreover, in addition to a lack of methodological standardization, the intrasubject variability of these functional assays upon repeated measurements is largely unexplored, making the routine interpretation of any single reading a questionable exercise, except perhaps in highly sophisticated academic centers.

Another clinically relevant dimension of PDs is related to the duration of drug target blockade. Both aspirin and thienopyridines are routinely given once daily. Every-other-day regimens of low-dose aspirin have been used with questionable success in two primary prevention trials. Thus, adequate drug receptor blockade must be maintained for approximately 24 hours. Although this is theoretically ensured by the irreversible mechanism of action of these antiplatelet drugs, very few studies have carefully monitored the persistency of the antiplatelet effect of aspirin or clopidogrel throughout the dosing interval. Moreover, most studies describing “resistance” to the antiplatelet effect of aspirin or clopidogrel were typically based on a single blood sample drawn at a variable (sometimes undefined) time interval after the last drug administration, making it hard to distinguish between incomplete drug target inactivation and faster renewal of the drug target as the mechanism potentially explaining inadequate platelet inhibition. We have recently characterized substantial interindividual variability in the rate of recovery of platelet COX-1 activity during the 12- to 24 hour dosing interval of low-dose aspirin administration in type 2 diabetes (Rocca et al, unpublished). Disorders of platelet production and/or destruction might be expected to alter the duration of the antiplatelet effect of both aspirin and thienopyridines, but not the PDs of reversible P2Y12 inhibitors for which the duration of the antiplatelet effect is dependent on the persistency of drug levels throughout the dosing interval, and the kinetics of drug dissociation from its receptor.

In summary, based on a PD analysis of variability in response to aspirin and P2Y12 blockers, there seems to be no solid grounds for the practice of phenotyping patients as being “resistant” or “nonresponders” to these drugs based on a single measurement of platelet function, performed at a variable time point after dosing, and using a largely arbitrary threshold response value. Moreover, unless the primary mechanism underlying a repeated finding of less-than-expected inhibition of platelet function at a standardized time point (e.g., 24 hours after a witnessed administration) is characterized in the individual patient, changing his/her antiplatelet therapy will represent a purely empirical exercise. Thus, while changing the daily dose of the drug is unlikely to overcome incomplete P2Y12 blockade in clopidogrel-
treated patients with inadequate metabolic activation of the prodrug, switching to a different thienopyridine (eg, prasugrel) or reversible P2Y12 blocker (eg, ticagrelor) may effectively address the problem. On the other hand, increasing the daily dose of aspirin or switching from clopidogrel to prasugrel is unlikely to overcome faster renewal of the drug target because of altered platelet production, a problem likely to require a change in the dosing interval of these irreversible antiplatelet agents or the administration of a reversible inhibitor.

**CLINICAL READOUTS OF VARIABLE COX-1 AND/OR P2Y12 BLOCKADE**

Aspirin typically reduces the risk of serious vascular events by approximately 20% to 30% in high-risk patients. In the CAPRIE (Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events) trial, clopidogrel 75 mg once daily displayed a comparable efficacy and safety profile as aspirin 325 mg once daily in a heterogeneous population of high-risk patients. Based on estimates of approximately 70% consistent medication adherence, these drugs could each be expected to avert one-quarter to one-third of all serious vascular events, reflecting the multifactorial nature of atherothrombosis and the important roles played by the TXA2/TP and ADP/P2Y12 axes as amplification mechanisms of platelet activation. As for the level of COX-1 blockade necessary to achieve this clinical benefit, this is presumably close to 100% on the following considerations: (i) serum TXB2 was suppressed by >99% in over 90% of a large series of consecutive aspirin-treated patients presenting for diagnostic cardiac catheterization; (ii) the relationship between inhibition of platelet COX-1 activity measured ex vivo and the reduction in urinary 11-dehydro-TXB2 excre-
tion (an in vivo index of TXA2-dependent platelet activation) is strikingly nonlinear, requiring 97% to 100% suppression of the former in order to maximally reduce the latter\(^8,39\); and (iii) reversible COX-1 inhibitors (eg, diclofenac, ibuprofen) that produce incomplete (70% to 90%) and transient blockade of TXA\(_2\) production share the same cardiotoxic phenotype of coxibs\(^40\) that only marginally inhibit platelet COX-1 activity.\(^21\)

As for the level of P2Y\(_{12}\) blockade necessary to achieve the clinical benefit produced by clopidogrel administration in the CAPRIE trial,\(^36\) this is more difficult to ascertain. Although we can speculate that even low levels of receptor blockade that produce incomplete inhibition of ADP-dependent platelet aggregation\(^33\) may be associated with a clinical effect, a more likely interpretation of the benefit of clopidogrel as monotherapy is that it represents an average value, resulting from a likely larger effect in the 70% noncarriers of CYP-polymorphism (producing adequate levels of the active metabolite) and a much lower or no effect in the 30% carriers of some loss-of-function CYP-polymorphisms (eg, CYP2C19*2) limiting formation of the active metabolite.\(^41\) When added to low-dose aspirin, clopidogrel produces a statistically significant additive clinical benefit, as would be expected from the complementary mechanism of action of the two drugs, in patients with acute coronary syndromes,\(^42\) ST-segment-elevation myocardial infarction,\(^43\) or atrial fibrillation,\(^44\) in the range of a 10% to 20% relative risk reduction of serious vascular events. Again, this relatively modest benefit is likely to represent an average value in the general population because of differentially responding patients. Faster, more profound, and less variable blockade of platelet P2Y\(_{12}\) may produce a larger benefit than that achieved by clopidogrel in acute coronary syndromes. This has now been convincingly established by two independent studies of prasugrel\(^26\) (Figure 3) and ticagrelor\(^45\) (Figure 4)\(^45,46\) demonstrating a 15% to 20% relative risk reduction in the primary end point versus clopidogrel. The genetic substudy of the TRITON-TIMI 38 (TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis In

![Figure 5. Mechanisms underlying interindividual variability in response to clopidogrel.](image)

Both pharmacogenetic (eg, reduced-function polymorphisms of cytochrome P450 isozymes) and pharmacokinetic (eg, drug interactions with inhibitors of CYP isozymes, such as some proton pump inhibitors) mechanisms may be responsible for inhibiting the two-step conversion of clopidogrel to its active metabolite. The resulting incomplete inactivation of platelet P2Y\(_{12}\) is associated with impaired inhibition of ADP-dependent platelet aggregation. In a pharmacogenetic substudy of the TRITON-TIMI 38 trial, clopidogrel-treated carriers of the reduced-function CYP2C19*2 allele had lower levels of the active metabolite of clopidogrel, diminished platelet inhibition, and a higher rate of major cardiovascular events than clopidogrel-treated noncarriers.

**Abbreviations:** ADP, adenosine diphosphate; CYP, cytochrome P450; PPI, proton pump inhibitor; TRITON-TIMI, Trials to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis In Myocardial Infarction.

Myocardial Infarction) 38 trial offers a quantitative assessment of the increased risk of major vascular events associated with the CYP2C19*2 polymorphism in clopidogrel-treated patients. Carriers of this loss-of-function genetic variant had an approximately 50% higher rate of major vascular events than noncarriers, who had a comparable event rate to that recorded in prasugrel-treated patients (Figure 5, page 13). The findings in TRITON-TIMI 38 have been confirmed, at least in part, by a similar genetic substudy of PLATO (PLATelet inhibition and patient Outcomes), but not by subanalyses of CURE (Clopidogrel in Unstable angina to prevent Recurrent Events) and ACTIVE A (Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events A) (Table I). Potential interactions between genetic polymorphisms and the effects of clopidogrel on clinical events seemed to be time dependent in PLATO, with significantly higher event rates in patients with versus those without any CYP2C19 loss-of-function allele at 30 days, but no significant difference between the genotype groups thereafter. Also, the variable rates of percutaneous coronary interventions performed in the TRITON-TIMI 38, PLATO, and CURE trials might contribute to the apparent heterogeneity in this pharmacogenetic association with clinical outcomes among the three studies in acute coronary syndromes. These interesting findings provide experimental support for the hypothesis

<table>
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<tr>
<th>Study</th>
<th>Population and treatment</th>
<th>Alleles under study</th>
<th>Primary end point</th>
<th>Outcome</th>
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</thead>
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<tr>
<td>Mega et al, 2009⁴¹</td>
<td>162 healthy subjects treated with clopidogrel 300, 600, or 75 mg</td>
<td>CYP2C19 loss- or gain-of-function variants, CYP2B6, CYP2C9, CYP3A4/5, CYP1A2</td>
<td>Inhibition of ADP-induced aggregation</td>
<td>Clopidogrel arm: 12.1% of primary outcomes in 395 patients with at least one 2C19 loss-of-function allele, 8% in 1064 noncarriers (HR, 1.53; 95% CI, 1.07-2.19). No effect of other genotypes</td>
</tr>
<tr>
<td>Wallentin et al, 2010⁴⁸</td>
<td>10 285 ACS patients from the PLATO trial, clopidogrel (300-600 mg LD, 75 mg MD) vs prasugrel (60 mg LD, 10 mg od MD)</td>
<td>CYP2C19 loss- or gain-of-function variants, ABCB1 3435C → T</td>
<td>CV death, MI, stroke</td>
<td>Clopidogrel arm: higher primary outcome incidence for any loss-of-function 2C19 allele only at 30 days (HR, 1.37; 95% CI, 1.04-1.82; P=0.028), but not at 1 year (11.2 vs 10%, P=0.25). 2C19 gain-of-function alleles associated with more major bleeds (11.9% vs 9.5%; P=0.022)</td>
</tr>
<tr>
<td>Parè et al, 2010⁴⁹</td>
<td>5059 ACS patients from the CURE trial, clopidogrel (300 mg LD, 75 mg MD) vs placebo</td>
<td>Two loss-of-function and one gain-of-function alleles of CYP2C19</td>
<td>CV death, nonfatal MI, or stroke</td>
<td>Clopidogrel arm: no difference in the primary end point between any loss-of-function allele and noncarriers (HR, 0.86; 95% CI, 0.63-1.17). Higher protection in gain-of-function carriers. No differences in bleeds</td>
</tr>
<tr>
<td></td>
<td>1156 AF patients from the ACTIVE-A trial, clopidogrel (75 mg od) vs placebo</td>
<td></td>
<td>Any major vascular event</td>
<td>Clopidogrel arm: no difference in the primary end point between any loss-of-function allele and noncarriers (HR 1.07; 95% CI, 0.7-1.63). No differences in bleeds</td>
</tr>
</tbody>
</table>

Table 1. Studies on CYP variants and clinical outcomes in randomized clinical trials of clopidogrel.

Abbreviations: ABCB, P-glycoprotein multidrug resistant-1 efflux transporter; ACS, acute coronary syndrome; ACTIVE A, Atrial fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events A; ADP, adenosine diphosphate; AF, atrial fibrillation; AUC, area under the curve; CURE, Clopidogrel in Unstable angina to prevent Recurrent Events; CV, cardiovascular; CYP, cytochrome; HR, hazard ratio; LD, loading dose; MD, maintenance dose; MI, myocardial infarction; PLATO, PLATelet inhibition and patient Outcomes; TRITON-TIMI, TRial to assess Improvement in Therapeutic Outcomes by Optimizing Platelet inhibition with prasugrel-Thrombolysis In Myocardial Infarction.
that the typical clinical benefit of clopidogrel in the general population indeed represents an average of heterogeneous clinical responses.

The information from randomized clinical trials that we have briefly reviewed above should provide an estimate of the pharmacologically plausible difference in clinical event rates between patients who are fully responsive to antiplatelet therapy and those who are poor responders. Given the effect size of aspirin monotherapy or clopidogrel added to aspirin, one would reasonably expect that the relative risk of poor responders to aspirin or clopidogrel may range between 1.3 and 1.5 (under a worst case scenario of no protective effects of the drug) as compared to good responders, assuming comparability of patient characteristics and adherence to prescribed therapy. Krasopoulos et al have reported a systematic review and meta-analysis of 20 studies that related a baseline diagnosis of aspirin “resistance” (based on a variety of biochemical and functional tests) to the risk of experiencing some sort of vascular complication, during follow-up of patients with cardiovascular disease. The sample size of these studies ranged between 28 and 326 patients. Approximately one quarter of these 2930 patients were phenotyped as being aspirin “resistant” at baseline.

The risk of experiencing any cardiovascular event was, on average, 4-fold (odds ratio [OR], 3.85; 95% confidence interval [CI], 3.08 to 4.80) higher in “resistant” as compared to aspirin “sensitive” patients, with individual odds ratios ranging between 0.87 and 142.3 In a similar meta-analysis of clopidogrel “nonresponsive- ness” and risk of cardiovascular morbidity, Sofi et al analyzed data from 14 studies (n=60 to 804) in which approximately one quarter of these 2930 patients were classified as “nonresponders.” Using a random-effects model for cardiovascular recurrences associated with poor response to clopidogrel at baseline, the overall odds ratio was 5.67 (95% CI, 2.97 to 10.84) with individual values ranging between 1.16 and 44.5. By excluding four outlier studies, the estimate of the association fell to 3.58 (95% CI, 2.54 to 5.05). If these numbers are real, then aspirin or clopidogrel “resistance” would represent the strongest predictor of cardiovascular morbidity, inasmuch as the typical odds ratio for the association of traditional risk factors with cardiovascular outcomes is rarely higher than 2.0.35

Alternatively, one could seek other explanations for these unexpected findings. These include: (i) misclassification of patients as “resistant” on “nonresponders” based on a single determination of platelet function using an arbitrary threshold value of response; (ii) cosegregation of genetic variants influencing PK/PD variability with genetic traits adversely affecting the natural history of atherothrombosis; and (iii) variable medication adherence in the two subgroups. It should be emphasized that compliance in these studies was typically ascertained by verbal interviews or questionnaires that largely underestimate the true rate of medication adherence.27 Nonadherence to medication has been documented to occur in over 60% of patients with cardiovascular disease.27 Self-reported medication adherence in these patients is <40% for the combination of aspirin, β-blocker, and statin and falls to 21% when it is based on more than two consecutive follow-up surveys over 6±12 months.27 Several studies have shown that both primary and secondary nonadherence lead to increased risk of cardiovascular events and mortality.27

In a single center, prospective study of 700 consecutive aspirin-treated patients presenting for coronary angiographic evaluation, residual platelet COX-1 activity (as reflected by serum TXB2) and COX-1–independent platelet function measured by PFA-100 collagen-ADP closure time, but not COX-1–dependent functional assays (eg, arachidonate-stimulated platelet markers), correlated with subsequent major cardiovascular events.38 Based on these findings the authors concluded that multiple mechanisms, including but not confined to inadequate inhibition of platelet COX-1, are responsible for poor clinical outcomes in aspirin-treated patients, and therefore the term “aspirin resistance” is inappropriate.38 The results of this study based on serum TXB2 determinations are consistent with the results of the two earlier studies, in which Eikelboom et al measured baseline urinary 11-dehydro-TXB2 excretion in a subgroup of aspirin-treated participants in the HOPE (Heart Outcomes Prevention Evaluation) and CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance) trials, and described an association of increasing risk for the primary end point with increasing quartiles of TXB2 metabolite excretion (odds ratio, 1.8 and 1.7 in each study, respectively, for the highest quartile relative to the lowest quartile).

The effect size is somewhat smaller and perhaps more realistic than in other studies based on functional measurements. Neither Frelinger’s study nor Eikelboom’s studies rigorously addressed the issue of medication adherence, although the former excluded two patients who had serum TXB2 in the range of aspirin-free healthy controls from follow-up. Of course, some of
<table>
<thead>
<tr>
<th>Study acronym</th>
<th>Study title</th>
<th>ClinicalTrials.gov identifier</th>
<th>Patients (n)</th>
<th>Outcome</th>
<th>Thienopyridine therapy</th>
<th>Randomization</th>
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<tbody>
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<td>GRAVITAS</td>
<td>Gauging responsiveness With A VerifyNow Assay- Impact on Thrombosis And Safety</td>
<td>NCT00645918</td>
<td>ACS-PCI-DES (2783)</td>
<td>6-month CV death, nonfatal MI, or ST</td>
<td>Clopidogrel 75 mg od vs 150 mg od or prasugrel 10 mg</td>
<td>Dose adjustment of clopidogrel among deemed nonresponders to 75mg of clopidogrel identified with the VerifyNow® POC</td>
</tr>
<tr>
<td>ARCTIC</td>
<td>Double Randomization of a Monitoring Adjusted Antiplatelet Treatment Versus a Common Antiplatelet Treatment for DES Implantation, and Interruption Versus Continuation of Double Antiplatelet Therapy</td>
<td>NCT00827411</td>
<td>Elective PCI-DES (2500)</td>
<td>12-month composite end point of death, MI, stroke, urgent revascularization, ST</td>
<td>Therapy based on maintenance test results</td>
<td>Use of the VerifyNow® POC for aspirin &amp; clopidogrel and subsequent dose adjustment in nonresponders versus standard care</td>
</tr>
<tr>
<td>DANTE</td>
<td>Dual Antiplatelet Therapy Tailored on the Extent of Platelet Inhibition</td>
<td>NCT00774475</td>
<td>Unstable or NSTEMI-PCI (442)</td>
<td>6- and 12-month CV death, nonfatal MI, TVR by PCI or CABG</td>
<td>Clopidogrel 75 mg od vs 150 mg od</td>
<td>Dose adjustment of clopidogrel among deemed nonresponders to 75 mg of clopidogrel identified with the VerifyNow® POC</td>
</tr>
<tr>
<td>TOPAS-1</td>
<td>Tailoring of Platelet Inhibition to Avoid Stent Thrombosis</td>
<td>NCT00914368</td>
<td>Previous PCI or stenting for CAD (450)</td>
<td>To establish cutoff level of platelet inhibition that separates patients with or without previous stent occlusion</td>
<td>Clopidogrel 600mg LD 75 mg od for 6 months</td>
<td>VerifyNow® P2Y12 (PRU)</td>
</tr>
<tr>
<td>TRIGGER-PCI</td>
<td>Testing Platelet Reactivity In Patients Undergoing Elective Stent Placement on Clopidogrel to Guide Alternative Therapy With Prasugrel</td>
<td>NCT00910299</td>
<td>2150</td>
<td>CV death, nonfatal MI</td>
<td>Prasugrel 60/10 mg vs clopidogrel 600/75 mg</td>
<td>Dose adjustment of clopidogrel among deemed nonresponders to 75 mg identified with the VerifyNow® POC</td>
</tr>
</tbody>
</table>

Table II. Ongoing trials of adjusted antiplatelet therapy in nonresponder patients.

Abbreviations: ACS, acute coronary syndrome; CABG, coronary artery bypass graft; CAD, coronary artery disease; CV, cardiovascular; DES, drug-eluting stent; LD, loading dose; MI, myocardial infarction; NSTEMI, non-ST-segment–elevation myocardial infarction; PCI, percutaneous coronary intervention; POC, point-of-care; PRU, P2Y12 reaction units; ST, stent thrombosis; TVR, target-lesion vessel revascularization.
the patients included may well have been irregularly noncompliant and displayed variable serum TXB2 levels outside the control range, but still in the nonfunctional range of platelet COX-1 inhibition (ie, <97%), depending on the time elapsed since the last aspirin intake. Moreover, these studies—by virtue of their design—did not provide insight into the mechanism(s) underlying incomplete inhibition of platelet COX-1 activity or “aspirin-resistant” TXA2 biosynthesis, nor did they assess the reproducibility of these abnormal biochemical phenotypes.

PERSPECTIVE AND CONCLUSIONS

The term “resistance” is uninformative of the mechanism(s) contributing to the interindividual variability in response to aspirin or clopidogrel, and is potentially misleading. Thus, it implies that drug response can be measured with a standardized method that has a direct bearing on clinical efficacy and, depending on its results, may lead to a change in antiplatelet therapy. In fact, as reviewed in this article, the relationship of the various functional indexes of platelet capacity that can be measured ex vivo to the actual occurrence of platelet activation and inhibition in vivo is far from established. Therefore, we and others have suggested that the term “resistance” should be abandoned in order to advance our understanding of the distinct determinants of the interindividual variability in response to aspirin or P2Y12 blockers that we have briefly discussed in the previous sections.

If measurements of platelet biochemistry and/or function during antiplatelet treatment are intended to provide complementary prognostic information to better define the cardiovascular risk of the patient, then we need a paradigm shift along the following lines: (i) standardizing the methods to be routinely employed; (ii) obtaining 2-3 repeated measurements from each subject to assess consistency of results; (iii) drawing blood samples at a fixed time interval (eg, 24 hours for aspirin and thienopyridines) after dosing; (iv) describing the results as being in the low, intermediate, or high range of response in the relevant patient population; and (v) relating these variable functional responses to the occurrence of cardiovascular and bleeding outcomes in adequately sized epidemiological studies.

For the impatient cardiologist unwilling to await the results of these additional studies, J. W. Eikelboom will address the question of current bedside monitoring of the antiplatelet response to aspirin and P2Y12 blockers, while D. J. Angiolillo will review the prevalence of “resistance” to these drugs.

If the same measurements are intended to provide guidance for tailoring antiplatelet therapy in the individual patient, then we need randomized clinical trials assessing the clinical effectiveness of testing versus nontesting in patients with inadequate response to standard regimens of antiplatelet therapy. Several ongoing trials are evaluating whether adjustment of antiplatelet therapy by use of functional thresholds defined to identify high versus low on-treatment platelet reactivity with aspirin or clopidogrel can improve efficacy while maintaining safety versus standard therapy (Table II). It should be acknowledged that both the size (442 to 2783 patients) and duration of follow-up (6 to 12 months) of these trials may be inadequate to detect relatively small differences between the two randomized strategies. A general strategy of using higher doses of aspirin, clopidogrel, or both in the early phase of acute coronary syndromes did not prove successful in the CURRENT-OASIS (Clopidogrel optimal loading dose Usage to Reduce Recurrent EveNTs-Organization to Assess Strategies in Ischemic Syndromes) trial. However, the ongoing trials are restricted to patients deemed to be poorly responsive to aspirin and/or clopidogrel, and some include prasugrel as a treatment option (Table II). For the impatient cardiologist unwilling to await the results of these trials, M. Valgimigli will address current therapeutic options for such poorly responsive, high-risk patients.

In conclusion, studies of “aspirin resistance” have led us to reexamine the clinical pharmacology of platelet COX-1 inhibition in health and disease. Serum TXB2 remains the gold standard test of response to aspirin, and its measurements clearly indicate noncompliance as well as a PD interaction with other NSAIDs as the main causes of incomplete COX-1 inhibition. The option of tailoring aspirin therapy to individual patients seems unnecessary, except perhaps for changing the pharmaceutical formulation (plain vs enteric-coated) and/or the dosing interval of aspirin administration where inadequate systemic bioavailability of the drug and/or accelerated renewal of the drug target can be identified as being responsible for less-than-complete inhibition of platelet COX-1. Studies of “clopidogrel resistance,” as well as the clinical development of prasugrel and ticagrelor, have been instrumental in characterizing the main determinants of the interindividual variability in response to P2Y12 blockers. Pharmacogenetic assessment of clopidogrel response has led to regulatory action that requires careful consideration. Ideally, tailoring P2Y12 blockade to the individual patient would require integrating PK/PD assessment with pharmacogenetics, inasmuch as the ther-
apeutic options might change depending on whether inadequate receptor blockade results from a PK interaction with concomitant therapy (eg, proton pump inhibitors [PPIs] or statins) or reflects genetically determined poor metabolic activation of the prodrug.

Monitoring and adjusting antiplatelet therapy according to a reliable, standardized PD response is as desirable as monitoring and adjusting lipid-lowering or antihypertensive therapy. Maintaining an adequate level of platelet inhibition throughout the dosing interval is probably as important as maintaining an adequate level of BP control. Although it is conceivable that some sort of platelet Holter monitoring will eventually become available, we have to realize that we are not there yet. But, the future looks bright.

Acknowledgments: The authors’ studies were funded by the European Commission (EICOSANOX FP6-Project). The expert editorial assistance of Ms. Daniela Basilico is gratefully acknowledged.

REFERENCES


Aspirin Resistance

Expert Answers to Three Key Questions

1

Is it possible to define resistance to acetylsalicylic acid and P2Y<sub>12</sub> blockers at the patient’s bedside?

*J. W. Eikelboom, G. J. Hankey*

2

What is the prevalence of resistance to aspirin and P2Y<sub>12</sub> blockers in high-risk patients?

*S. D. Tomasello, D. J. Angiolillo*

3

How do you overcome aspirin and P2Y<sub>12</sub> blocker resistance in secondary prevention in high-risk patients?

*A. Scalone, M. Valgimigli*
Is it possible to define resistance to acetylsalicylic acid and P2Y12 blockers at the patient’s bedside?

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Graeme J. Hankey, MBBS, MD, FRACP, FRCP†

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Multiple assays exist to measure antiplatelet effects of acetylsalicylic acid and clopidogrel, and numerous studies have demonstrated an association between incomplete inhibition with these drugs and risk of cardiovascular events. Simple point-of-care assays are potentially suitable for bedside use, and cutoffs for antiplatelet drug “resistance” diagnosis have been proposed. However, many assays remain poorly standardized and validated, different assays correlate poorly with one another, and there is uncertainty whether resistance is a continuous or a categorical variable. In addition, randomized trials are needed to show that altering therapy based on assay results is beneficial. Routine bedside testing for antiplatelet drug resistance is therefore not recommended until these issues have been resolved.

Keywords: aspirin; clopidogrel; platelets; resistance; cardiovascular; aggregation

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The demonstration of an association between incomplete inhibition by an antiplatelet drug of its therapeutic target and increased risk of cardiovascular events raises the potential for treatment with additional or alternative therapies that could prevent future cardiovascular events in affected individuals. For such a strategy to be successful we need to be able to reliably identify patients who are “resistant” to ASA or clopidogrel, who can then be targeted with more effective treatments.

In this paper we critically examine the definition of antiplatelet drug resistance and explore whether it is possible to identify patients who are resistant to ASA or clopidogrel at the bedside.

WHAT IS ANTIPLATELET DRUG RESISTANCE?

The term “resistance” is most commonly used in medicine to describe resistance by a pathogen to an antimicrobial drug or by tumor cells to a chemotherapeutic agent. Antimicrobial and chemotherapeutic drug resistance generally develop in response to drug exposure and are characterized by a reduction in effective drug concentrations resulting from: (i) enhanced drug efflux or increased rates of drug metabolism reducing inhibition of the target; or (ii) a change in the drug target that makes the cell less susceptible to the effects of the drug; or (iii) the evolution of a mechanism that negates the impact of the drug.

“Antiplatelet drug resistance” is a term that is used to refer to the suboptimal inhibition of its target by an antiplatelet drug that correlates
causally with an increased risk of cardiovascular events. In the case of ASA, the target is platelet cyclooxygenase, while in the case of clopidogrel and other P2Y12 receptor antagonists, the target is the P2Y12 receptor on the surface of platelets.

As with many drugs, antiplatelet response to ASA and P2Y12 blockers, such as clopidogrel, varies according to patient age and body weight and is affected by differences in patient adherence, drug dose, gastrointestinal absorption, drug metabolism, and rates of platelet activation and turnover, as well as cigarette smoking, drug interactions, and comorbid conditions such as diabetes and hypercholesterolemia (Table I). However, there are important differences between antiplatelet drug resistance and resistance to antimicrobial or chemotherapeutic agents. Firstly, unlike resistance to antimicrobial therapy or chemotherapy, there is no evidence that exposure to ASA or clopidogrel promotes the development of antiplatelet drug resistance. Secondly, none of the reported mechanisms of antiplatelet drug resistance involve resistance of the drug target to the actions of the drug. Thirdly, the mechanisms of antiplatelet drug resistance can potentially be overcome in most cases by

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<th>Test</th>
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<th>Advantages</th>
<th>Limitations</th>
<th>Drugs that can be monitored</th>
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<tr>
<td>VerifyNow®</td>
<td>Agglutination of fibrinogen-coated beads</td>
<td>Simple, low sample volume, no sample preparation, rapid, and objective end point, and best-evaluated assay to date</td>
<td>Affected by vWF level and function, hematocrit, and platelet count and requires sample pipetting</td>
<td>Aspirin and ADP receptor antagonists</td>
</tr>
<tr>
<td>PFA-100</td>
<td>Aperture occlusion by platelet plug under high-shear conditions</td>
<td>Simple, low sample volume, no sample preparation, rapid, and objective end point</td>
<td>Analysis needs to be performed within 10 min of sample collection</td>
<td>Aspirin</td>
</tr>
<tr>
<td>PlateletWorks</td>
<td>Platelet count pre- and postactivation with agonist (ADP, collagen, and AA)</td>
<td>Simple, low sample volume, no sample preparation, rapid, and provides platelet count</td>
<td>Requires manual addition of reagents and sample pipetting, and interpretation of results requires expertise</td>
<td>Aspirin and ADP receptor antagonists</td>
</tr>
<tr>
<td>TEG-PM</td>
<td>Quantification of the rate and strength of clot formation</td>
<td>Provides measure of global hemostasis</td>
<td>Requires sample pipetting and limited evaluation</td>
<td>Aspirin and ADP receptor antagonists</td>
</tr>
<tr>
<td>Impact cone and platelet analyzer</td>
<td>Quantification of platelet adhesion under high-shear conditions</td>
<td>Low sample volume and rapid results</td>
<td></td>
<td>Aspirin and ADP receptor antagonists</td>
</tr>
</tbody>
</table>

Table II. Point-of-care tests available for the monitoring of antiplatelet therapy.

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; PFA, platelet function analyzer; TEG-PM, thromboelastography platelet mapping; vWF: von Willebrand factor.

improving adherence, increasing the dose of the drug or frequency of administration, or by avoiding interacting drugs. We believe that “antiplatelet response variability” is a better term than “antiplatelet drug resistance” to describe the association between incomplete inhibition by ASA or clopidogrel of their targets and increased risk of cardiovascular events.

Antiplatelet drug resistance has also been used to describe the occurrence of atherothromboembolic cardiovascular events in patients who are prescribed ASA or clopidogrel (ie, aspirin “failures”). However, we believe that the term should not be used in this context. The occurrence of arterial vascular events despite treatment with effective antiplatelet therapies is not surprising because cardiovascular disease is multifactorial, and drugs that target a single pathway of platelet activation cannot be expected to prevent all vascular events. Labeling patients who experience a cardiovascular event while prescribed ASA or clopidogrel as resistant implies that they are not benefiting from antiplatelet therapy, but the results of randomized controlled trials confirm that ASA and clopidogrel prevent only a minority of cardiovascular events. Whilst it might still be appropriate to prescribe a more effective antiplatelet drug to patients who experience a cardiovascular event despite taking ASA or clopidogrel, it would seem inappropriate to label such patients “resistant” (or poorly responsive) to ASA or clopidogrel simply because they experience a cardiovascular event.

### IS IT POSSIBLE TO DEFINE RESISTANCE TO ACETYLSALICYLIC ACID AND P2Y12 BLOCKERS AT THE PATIENT’S BEDSIDE?

Numerous biochemical and platelet function assays have been developed to measure the effect of ASA and clopidogrel on their target. Furthermore, simple, convenient, standardized, point-of-care assays that are suitable for use at the bedside are now available and have been shown to yield reproducible results (Table II).8

Observational studies have consistently demonstrated that less complete suppression of biochemical markers of platelet activation or of platelet function as measured by one or more of these assays inde-

<table>
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<tr>
<th>Study (year)</th>
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<th>Assays used to establish platelet responsiveness</th>
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<tr>
<td>Snoep et al (2007)9</td>
<td>16</td>
<td>1813</td>
<td>LTA, plasma or serum TXB2, PFA-100, VerifyNow®, bleeding time, platelet reactivity index, and TEG</td>
<td>Aspirin</td>
<td>CV outcomes (defined as: CV death, MI, strokes, ACS, revascularization, myonecrosis, reocclusion after bypass or angioplasty): OR, 3.8; 95% CI, 2.3-6.1</td>
</tr>
<tr>
<td>Snoep et al (2007)10</td>
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<td>3688</td>
<td>Flow cytometry, LTA, and electrical aggregation</td>
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</tr>
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<td>Crescente et al (2008)11</td>
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<td>3003</td>
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<td>CV events (not defined): OR, 2.35; 95% CI, 1.96-2.83</td>
</tr>
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<td>Krasopoulos et al (2008)12</td>
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<td>2930</td>
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<td>CV events (defined as: CV death, stroke, ACS, or failure of vascular intervention): OR, 3.85; 95% CI, 3.08-4.8</td>
</tr>
<tr>
<td>Reny et al (2008)13</td>
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<td>PFA-100</td>
<td>Aspirin</td>
<td>Ischemic events (not defined): OR, 2.1; 95% CI, 1.4-3.4</td>
</tr>
<tr>
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<td>11</td>
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<td>LTA, PFA-100, and propyl gallate-induced aggregation</td>
<td>Aspirin</td>
<td>Clinical CV recurrences (not defined): RR, 3.11; 95% CI, 1.88-5.15</td>
</tr>
</tbody>
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Table III. Summary of meta-analysis of studies linking antiplatelet drug resistance with clinical outcome.

Abbreviations: ACS, acute coronary syndrome; CV, cardiovascular; LTA, light transmission aggregometry; MI, myocardial infarction; PFA, platelet function analyzer; TEG, thromboelastography; TXB2, thromboxane B2; WB, whole blood.

pendently predicts the risk of cardiovascular events in patients treated with ASA or clopidogrel (Table III, page 25).8-14

However, important limitations remain. A single cutoff to define resistance has been proposed for several assays of platelet function, but it seems likely that resistance is a continuous phenomenon, as evidenced by a normal distribution of platelet response to clopidogrel and, to a lesser extent, aspirin, and a progressive increase in cardiovascular risk with increasing degrees of resistance.3,4 The results of different tests for ASA or clopidogrel resistance, respectively, correlate poorly with one another and patients defined as being resistant with one assay might not be defined as being resistant using another.15,16

It is possible that different assays for antiplatelet drug resistance yield complementary information concerning cardiovascular risk, but this remains uncertain. Finally, there is no convincing evidence to date that a strategy of changing treatment based on the results of testing compared with standard care improves clinical outcomes, although several trials testing this hypothesis are currently ongoing.8

Genetic testing also has the potential to identify patients who are resistant to antiplatelet therapy.17 Carriers of two cytochrome P450 (CYP) 2C19 loss-of-function alleles (so-called ‘poor metabolizers’) that have reduced conversion rates of clopidogrel to its active metabolite and an increased risk of cardiovascular disease can be identified rapidly by new technologies that do not share many of the limitations of biochemical and functional platelet testing.18 The Food and Drug Administration recently issued a black box warning indicating the reduced effectiveness of clopidogrel in patients who are carriers of CYP 2C19 loss-of-function alleles and has recommended that affected individuals receive a higher dose of clopidogrel or an alternative antiplatelet agent.19 However, the 1.5- to 5-fold excess cardiovascular risk reported in clopidogrel-treated carriers of CYP 2C19 loss-of-function alleles18 seems implausibly large and does not appear to impact the benefits of clopidogrel relative to placebo,20 raising the possibility that the association is confounded, for example, by linkage disequilibrium with other genetic markers that predict cardiovascular risk. Furthermore, genetic testing is expensive, not widely available, and it remains to be demonstrated in ongoing trials whether modifying treatment based on the results of genetic testing improves clinical outcomes.

**CONCLUSION**

Antiplatelet drug resistance can be diagnosed at the bedside using commercially available point-of-care assays, but the tests correlate poorly with one another, and there is uncertainty about the optimal test and the appropriate cutoff to define resistance. Ultimately it is only worthwhile trying to define resistance to ASA or clopidogrel at the patient bedside using laboratory testing if the test reliably identifies at-risk individuals and if the assay results prompt a change in clinical management that reduces the incidence of recurrent vascular events with an acceptable safety profile and cost. Trials are currently ongoing to evaluate the impact of routine testing and whether modification of therapy based on the results of testing leads to improvements in clinical outcome. Until these data are available, we believe that it is premature to advocate the use of routine bedside testing.

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What is the prevalence of resistance to aspirin and P2Y_{12} blockers in high-risk patients?

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Despite the clinical benefits associated with therapy with aspirin, clopidogrel, or a combination of both, numerous patients continue to experience atherothrombotic events. Evidence suggests this may be due to inadequate suppression of platelet activation, “antiplatelet drug resistance.” The prevalence of antiplatelet drug resistance varies considerably in the literature and is related to differences in the definitions adopted, the type of assay used, the drug dosage, and the patient population. High-risk patients, such as those with diabetes mellitus or presenting with an acute coronary event, often have heightened platelet reactivity and an increased prevalence of antiplatelet drug resistance. The aim of this article is to provide insights into antiplatelet drug resistance with a particular focus on how this phenomenon affects high-risk patients.

P latelet-mediated thrombosis is a pivotal process in atherothrombosis and its complications. This fact underlines the importance of platelet-inhibiting strategies in patients with atherosclerotic disease manifestations in reducing the risk of recurrent atherothrombotic events. Platelet-mediated thrombosis is comprised of three phases: adhesion, activation, and aggregation. Each of these phases represents a potential therapeutic target for the prevention of recurrent ischemic events. Currently, no platelet adhesion inhibitors have been shown to be clinically effective, while inhibitors of platelet aggregation (glycoprotein IIb/IIIa inhibitors) are available only for parenteral use, thus limiting their use to the acute treatment phases. Therefore, practitioners rely on the use of platelet activation inhibitors for both acute and long-term prevention of recurrent ischemic events.

Currently approved inhibitors of platelet activation include aspirin, an inhibitor of the cyclooxygenase-1 (COX-1) enzyme, and P2Y_{12} receptor blockers appertaining to the family of thienopyridines (ticlopidine, clopidogrel, prasugrel). Clopidogrel is currently the most broadly utilized P2Y_{12} receptor blocker, with the most clinical and laboratory data available. Treatment with aspirin and clopidogrel represents standard care for the prevention of recurrent ischemic events in high-risk patients.

SELECTED ABBREVIATIONS AND ACRONYMS

| ACS | acute coronary syndrome |
| ADP | adenosine diphosphate |
| ASPECT | ASpirin-induced Platelet EffeCT |
| CHARISMA | Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance [trial] |
| COX-1 | cyclooxygenase 1 |
| CYP | cytochrome P450 |
| DES | drug-eluting stent |
| DM | diabetes mellitus |
| LTA | light transmittance aggregometry |
| PCI | percutaneous coronary intervention |
| RECLOSE | low REsponsiveness to CLOpidogrel and Sirolimus- or Paclitaxel-Eluting stent thrombosis [trial] |
| STEMI | ST-segment–elevation myocardial infarction |

Keywords: aspirin; P2Y_{12} blocker; resistance; high-risk patient

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in particular those presenting with an acute coronary syndrome (ACS) and undergoing percutaneous coronary intervention (PCI). The benefit of these agents increases as the degree of atherothrombotic risk of the patient increases. However, despite the clinical benefits associated with therapy with aspirin, clopidogrel, or a combination of both, a considerable number of patients continue to experience atherothrombotic events. Emerging evidence suggests that this may, in part, be attributed to inadequate suppression of platelet activation, known as “antiplatelet drug resistance.” The aim of the present article is to provide insights into antiplatelet drug resistance with a focus on how this phenomenon affects high-risk patients, in particular those with diabetes mellitus (DM) and those presenting with an ACS.

**ANTIPLATELET DRUG RESISTANCE: DEFINITIONS**

Antiplatelet drug resistance has the following laboratory-based definition: the failure of an antiplatelet agent to block its specific target. This implies that specific laboratory tests are required to assess whether a given antiplatelet agent is adequately inhibiting its target, such as the COX-1 enzyme for aspirin or the P2Y12 receptor for clopidogrel. Thus, the occurrence of an atherothrombotic event despite the use of aspirin and/or clopidogrel therapy should not be considered as synonymous with “resistance.” Rather, this should be considered “clinical treatment failure.” In fact, the existence of clinical resistance is extremely difficult to prove because multiple reasons that explain the ineffectiveness of an antiplatelet agent may exist (Figure 1).

There are several possible explanations for the lack of effect of aspirin therapy. It is well recognized that platelets can be activated by a multitude of pathways that are not blocked by aspirin. Also, a specific aspirin regimen may not achieve an optimal antithrombotic effect in some patients. Moreover, some patients may be able to generate thromboxane A2 despite standard aspirin treatment regimens; therefore, they fail to benefit from aspirin treatment. Finally, drug-drug interactions (eg, ibuprofen increases the risk of bleeding with both aspirin and clopidogrel), individual pharmacokinetic variations, and some genetic polymorphisms may decrease the antiplatelet efficacy of aspirin. Similarly, multiple etiologies for poor clopidogrel responsiveness have been proposed, including reduced drug bioavailability, alterations in the activity of hepatic cytochrome P450 (CYP) isoenzymes caused by drug-drug interactions between clopidogrel and different classes of drugs (eg, CYP2C19-specific proton pump inhibitors, such as omeprazole), and some CYP450 genetic polymorphisms and variations in P2Y12 receptor density. It is important to underscore that the role of the above mentioned factors implies that the patient is compliant to a specific antiplatelet drug regimen. In fact, noncompliance represents a common and underestimated cause of antiplatelet drug resistance.

**PREVALENCE OF ASPIRIN RESISTANCE**

The prevalence of aspirin resistance reported in the scientific literature varies considerably in relation to the definition adopted, the type of assay used, the dose of aspirin, and the patient population considered. However, using tests that specifically assess COX-1 activity (eg, serum
thromboxane B₂ or light transmission aggregometry (LTA) using stimuli with arachidonic acid, aspirin only produces resistance infrequently, while its prevalence increases when tests that are not specific for COX-1 activity are used. While different assays testing aspirin sensitivity (specific and nonspecific for COX-1 inhibition) are currently being evaluated in more depth, several studies, including meta-analyses that use these findings, results found using the same platelet test by demonstrating a reduced platelet response to aspirin. A recent study confirmed these findings using the same platelet test by demonstrating a rate of aspirin resistance of 23% in 48 consecutive type 2 DM patients. Moreover, this study found a significant association between aspirin resistance, levels of HbA₁c >8% (odds ratio [OR], 16; 95% confidence interval [CI], 3 to 94, P<0.002), and obesity (OR, 7; 95% CI, 1 to 32; P=0.02), which underscore that a lack of glycemic control in DM patients may promote high platelet reactivity. In agreement with these findings, results found using the impedance aggregometer multiplate analyzer with collagen and adenosine diphosphate (ADP) agonists demonstrated, in 175 patients, that aspirin resistance was higher in DM patients and was positively correlated with HbA₁c levels and obesity.

Figure 2. The prevalence of aspirin resistance as determined by various platelet assays. Aspirin resistance was defined as ≥20% platelet aggregation by light transmission aggregometry (LTA), an impedance >3MΩ for whole-blood aggregometry (WBA), a microscopic aperture closure time <193 s for PFA-100® (Dade International Inc), an aspirin reaction ≥550 U for the VerifyNow® (Accumetrics Inc) aspirin point-of-care system, and individuals with urinary 11-dehydrothromboxane B₂ (dTXB₂) levels ≥67.9 ng/mmol for the urinary dTXB₂ assay. Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; dTXB₂, 11-dehydrothromboxane B₂; LTA, light transmission aggregometry; WBA, whole-blood aggregometry. Modified from reference 24: Lordkipanidze et al. Eur Heart J. 2007;28:1702-1708. © 2007, The European Society of Cardiology.
Eikelboom et al demonstrated that in high-risk aspirin-treated patients elevated urinary levels of 11-dehydrothromboxane B2, and consequently aspirin low-responsiveness, were associated with a twofold increase in myocardial infarction frequency and 3.5-times the risk of cardiac death. These results were confirmed in a recent prespecified subanalysis of the CHARISMA (Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance) trial. Moreover, in this substudy, the addition of clopidogrel to the treatment of aspirin low-responders did not reduce urinary 11-dehydrothromboxane B2 levels or the hazard ratio of cardiovascular events.

Recently, Valles et al evaluated the effect of aspirin on platelet reactivity in patients with ST-segment-elevation myocardial infarction (STEMI) with respect to thromboxane A2 ex vivo synthesis. They found a partial aspirin effect in 34% of patients, with higher peaks of troponin T and creatine kinase serum levels, which led to an increase in myonecrosis.

### PREVALENCE OF P2Y12 RESISTANCE

As with aspirin, the prevalence of clopidogrel resistance reported in the literature varies considerably and is related to differences in the adopted definitions, the type of assay used, the dose of clopidogrel, and the patient population. Nonetheless, the interindividual variability of platelet response to clopidogrel is a well-established concept, with some patients having either a poor response or no response to this therapy (Figure 4, page 33).

Numerous studies using various platelet function tests support the prognostic implications of this phenomenon (Table I, page 32).

Clopidogrel nonresponsiveness is more prevalent in patients with DM than in nondiabetic patients. Numerous mechanisms may account for this observation, however, up-regulation of the P2Y12 pathway appears to be of particular importance in patients with type 2 DM. In vitro
Prevalence of aspirin and P2Y₁₂ blocker resistance in high-risk patients - Tomasello and Angiolillo

<table>
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<tr>
<th>Research group</th>
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<td>Matetzky et al</td>
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<td>Postprimary PCI ischemic events (6 months)</td>
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<td>Gurbel et al</td>
<td>Nonemergent PCI</td>
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<td>Gurbel et al</td>
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<tr>
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<td>Cuisset et al</td>
<td>NSTEACS undergoing PCI</td>
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<td>Müller et al</td>
<td>MI undergoing PCI</td>
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<tr>
<td>Buonamici et al</td>
<td>PCI with drug eluting stent</td>
<td>Stent thrombosis</td>
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</table>

| **VASP-P (vasodilator-stimulated phosphoprotein phosphorylation) assay** | | |
| Bonello et al | Stable angina and low-risk NSTEACS undergoing PCI | Post-PCI major adverse cardiac events (6 months) |
| Frere et al | NSTEACS undergoing PCI | Post-PCI ischemic events (30 days) |
| Barragan et al | Subacute stent thrombosis | Stent thrombosis |
| Gurbel et al | Subacute stent thrombosis | Stent thrombosis |
| Blindt et al | PCI with high risk for stent thrombosis | Stent thrombosis |

| **VerifyNow® P2Y₁₂ assay** | | |
| Price et al | PCI with drug eluting stents | Major adverse cardiac events and stent thrombosis (6 months) |
| Patti et al | PCI | Major adverse cardiac events (30 days) |
| Marucci et al | ACS undergoing PCI | Major adverse cardiac events (12 months) |
| De Miguel et al | NSTEACS undergoing coronary angiography | Major adverse cardiac events (12 months) |

| **Others** | | |
| Sibbing et al* | Elective PCI with drug eluting stent | Stent thrombosis |
| Ajzenberg et al† | Subacute stent thrombosis | Stent thrombosis |

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Table 1. Clinical outcomes and inadequate clopidogrel response defined according to various platelet function assays.

Abbreviations: ACS, acute coronary syndrome; NSTEACS, non-ST-segment–elevation acute coronary syndrome; PCI, percutaneous coronary intervention; STEMI, ST-segment–elevation myocardial infarction.

studies have shown that insulin reduces platelet aggregation by inhibiting the P2Y12 pathway and interacting with its own receptor on the surface of the platelet leading to loss of G-protein–coupled receptor G, activity.43 This results in suppression of cAMP, inhibition of P2Y12 signaling, and reduced platelet reactivity. However, platelets of DM patients are victims of the insulin resistance phenomenon, which results in decreased sensitivity to insulin.40 The net result is upregulation of the P2Y12 pathway and increased platelet reactivity in DM patients. A previous study demonstrated lower clopidogrel responsiveness in type 2 DM patients include increased exposure to ADP, increased cytosolic levels of calcium, increased platelet turnover, and impaired renal function.41,45 Variability in response may exist even within this high-risk cohort of patients with DM, in which those who display highest on-treatment platelet reactivity have the highest long-term risk of recurrent events.46

Soffer et al studied the impact of angina class on the inhibition of platelet aggregation following clopidogrel administration and found that patients with unstable angina have a lower inhibition of platelet aggregation than stable angina patients.47 More recently, further studies have confirmed that patients with ACS have a higher prevalence of poor clopidogrel response and, within this cohort, those identified as low-responders also have an increased risk of cardiovascular events.48,49 The effect of clopidogrel resistance on patients with acute myocardial infarction was studied by Matetzky et al.50 They found clopidogrel resistance in up to 25% of STEMI patients undergoing primary PCI, which demonstrated an association between clopidogrel low-responsiveness and recurrent atherothrombotic cardiovascular events, during a 6-month follow-up.45 This underscores the need for alternative and more potent P2Y12 receptor inhibiting agents. Several novel P2Y12 receptor antagonists are currently being developed.51 Among these, two oral agents (prasugrel and ticagrelor) used in both the acute and chronic phases of treatment have recently completed phase 3 clinical investigation.52,53 Both prasugrel and ticagrelor are characterized by increased potency and are associated with less response variability and a reduced prevalence of pharmacodynamic resistance compared to clopido-
These agents have been shown to be associated with a reduced risk of recurrent ischemic events, including stent thrombosis, in ACS patients, which supports the hypothesis that greater platelet inhibition in these high-risk settings can improve outcomes, albeit at the expense of increased bleeding. Studies in a real-world setting are warranted to better define the prevalence of pharmacodynamic resistance, if any, with these novel compounds.

PREVALENCE OF COMBINED ASPIRIN AND P2Y12 RESISTANCE

The first evidence of combined aspirin and clopidogrel resistance was reported by Lepantalo et al in 2004.56 In a group of 50 patients undergoing PCI, 5 (10%) were found to be “poor responders” to both aspirin (using PFA-100® and LTA) and clopidogrel (using LTA). Although a limitation of this study was the small number of patients, it did suggest that a subgroup of patients might have a low response to both drugs. There are several plausible explanations for this phenomenon; however, the most likely mechanism is related to a “global” increase of platelet reactivity with a hyperresponse to multiple agonists.57

Indeed, platelets from aspirin-resistant patients appear to have increased sensitivity to other agonists.58,59 Furthermore, patients with diabetes, who comprised more than half of the aspirin-resistant group, have been shown to have a higher proportion of platelets expressing P-selectin and activated glycoprotein Iib/IIIa receptors than nondiabetic patients.60 In another study including patients undergoing PCI (n=150), Lev et al demonstrated that aspirin-resistant patients had a significantly lower degree of platelet aggregation reduction in response to 5 and 20 µmol/L ADP after clopidogrel administration.61 They found a total rate of combined resistance to both aspirin and clopidogrel of 6%. These patients had a greater increase in the markers of myocardial infarction after PCI compared to other subjects, and dual antiplatelet resistance was more likely to be identified in women. Recently, a subanalysis of the RECLOSE (low REsponsiveness to CLOpidogrel and Sirolimus- or Paclitaxel-Eluting stent thrombosis) trial62 (n=746) demonstrated that dual nonresponsiveness to aspirin and clopidogrel, although relatively infrequent (6%), identified patients at high risk of drug-eluting stent (DES) thrombosis (hazard ratio [HR], 5.31; 95% CI, 1.93 to 14.60; \( P = 0.001 \)) and the composite end point of death and DES thrombosis (HR, 2.94; 95% CI, 1.16 to 7.41; \( P = 0.022 \)).

CONCLUSION AND FUTURE PERSPECTIVES

Poor responsiveness to antiplatelet agents is an emerging clinical entity. The prevalence of this phenomenon varies considerably in relation to the adopted definition, the type of assay used, and the population considered. Although there is increasing evidence that monitoring the effects of antiplatelet therapy may identify patients at an increased risk of developing ischemic events,
current clinical guidelines do not support routine screening for antiplatelet drug response. Currently, there are multiple assays available to test for antiplatelet drug response, but many of these are expensive, time-consuming, and not broadly available. Therefore, rapid and accurate diagnosis of the responsiveness to antiplatelet agents also remains an issue, and widespread clinical application of the assessment of antiplatelet drug responses will require studies in large populations. These will have to define responsiveness in a standardized manner using assays (eg, intensification or reduction of antithrombotic medication in patients with hypo- and hyperresponsiveness, respectively) will potentially reduce ischemic and bleeding risks. Results from current, ongoing, large-scale clinical trials are awaited to define the safety and efficacy of this strategy.

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*J Am Coll Cardiol.* 2008;52:734-739.
Dual antiplatelet therapy is the cornerstone of treatment for patients with acute coronary syndrome (ACS) in secondary prevention. Many studies have shown that patients with a poor response to clopidogrel and/or aspirin are at increased risk of death, reinfarction, and stent thrombosis. Several methods have been used to assess the effects of clopidogrel and aspirin on platelets, and various techniques have been used to identify a poor response to dual antiplatelet therapy. This paper focuses on strategies to overcome poor responses to aspirin and/or clopidogrel. While several therapeutic options have been associated with an improved mechanistic response to both drugs, only a limited number have concomitantly improved clinical outcomes in patients with high on-treatment platelet reactivity after ingestion of aspirin or clopidogrel. Several clinical trials are in progress to determine whether tailoring antiplatelet therapy to individual residual platelet reactivity is worthwhile.

**Keywords:** aspirin; P2Y12 blocker; resistance; secondary prevention; high-risk patients

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**How to Overcome Aspirin Resistance**

Aspirin is a first-line oral antiplatelet drug used to prevent thromboembolic arterial occlusions. It irreversibly inhibits the cyclooxygenase 1 (COX-1) enzyme by acetylating a serine residue, thereby preventing the conversion of arachidonate to thromboxane A2 (TXA2), a vasoconstrictor and a promoter of platelet aggregation. Although aspirin is an efficacious therapy, a considerable number of patients who take it continue to experience atherothrombotic complications. This raises the question of whether recurrences of atherothrombotic complications while patients are taking the drug reflect poor responsiveness to treatment. The issue of "aspirin resistance" has been intensely investigated in the past, yet its definition and even its existence remain uncertain. Direct comparisons of various laboratory methods used to detect aspirin resistance have shown relatively weak or no correlation, which indicates that they are sensitive to different parameters. Indeed, studies that measured serum thromboxane B2 (TXB2) levels in aspirin-treated patients reported a prevalence of aspirin resistance that ranged from 1% to 1.7%. Therefore, aspirin resistance, when tested appropriately with COX-1 specific assays, appears to be rare and may instead reflect underdosing or non-compliance issues. Aspirin resistance is probably best assessed through the quantification of TXA2 production, either by measurement of serum TXB2 or urinary excretion of TXB2 metabolites. Schwartz et al
investigated the issue of compliance associated with long-term aspirin therapy. They demonstrated that lack of drug compliance can falsely indicate aspirin resistance. They found that there was an 11% increase in platelet inhibition when aspirin intake was witnessed as opposed to when it was verbally assessed. Moreover, drug interactions may also play a role. Nonsteroidal anti-inflammatory drug (NSAID) ingestion can blunt platelet inhibition during long-term aspirin therapy. There is abundant evidence that regular, but not intermittent, use of NSAIDs inhibits the clinical benefits of aspirin. Finally, the aspirin regimen itself is also likely to play a critical role. Previous data suggest that biological platelet resistance to low-dose aspirin frequently occurs in diabetic patients in secondary prevention. The higher platelet turnover described in this population could contribute to the reduced effect of aspirin on platelets. Recently, Drouet et al assessed the rate of biological aspirin resistance in diabetic patients shortly after aspirin ingestion (peak effect) compared with the rate 24 hours after aspirin was last taken. Fifty-two type 2 diabetic patients with stable coronary artery disease taking 75 mg (n=21) or 100 mg (n=31) of aspirin daily for at least 10 days were enrolled in the study. Platelet aggregation was induced with arachidonic acid (AA) and closure time was measured with the Platelet Function Analyzer-100® 2 and 24 hours after aspirin was last taken. Aspirin resistance was defined as a residual aggregation \( \leq 20\% \) with 0.5 mg/mL AA. The results show that all patients were sensitive to aspirin 2 hours after ingestion; however, 16 patients (31%) were aspirin-resistant 24 hours after aspirin was last taken. Forty-eight percent of patients in the 75 mg group and 23% in the 100 mg group showed poor response to the drug at the later assessment. Concordant results were found in other studies that did not use the PFA-100®. Hence, low-dose aspirin may not offer stable 24-hour biological protection in a high proportion of diabetic patients in secondary prevention. Whether this finding has clinical implications is subject to debate.

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The hypothesis that a high-dose aspirin regimen is associated with fewer cardiovascular events was formally tested in the CURRENT-
OASIS (Clopidogrel optimal loading dose Usage to Reduce Recurrent EveNTs–Organization to Assess Strategies in Ischemic Syndromes) 7 trial, in which a large number of ACS patients (25,087) were randomly allocated to either low- (75 or 100 mg/day) or high- (300 or 325 mg/day) dose aspirin regimens for 30 days. While the ischemic composite end point did not differ (4.7% in the low- vs 4.7% in the high-dose regimen group; P=0.44), there was a trend toward increased gastrointestinal bleeding (P=0.051) in the high-dose aspirin group. Based on reference 7.

Supporters of the “more is better” concept should remember that increasing aspirin dosage to greater than 100 mg/day has been shown to increase bleeding complications without a concomitant reduction in cardiovascular end points.8 A more appealing strategy is to divide the daily aspirin dosage in two (ie, rather than taking aspirin 100 mg in the morning, taking aspirin 50 mg twice daily). This may cause inhibition of platelet aggregation to become more stable over 24 hours without increasing the risk of bleeding complications. However, this hypothesis requires formal testing in clinical trials.

Another appealing strategy was recently suggested by Komowsky et al. This strategy states that omega-3 fatty acids decrease the availability of platelet AA and, indirectly, TXA₂ formation. The authors enrolled 485 subjects with stable coronary artery disease taking low-dose aspirin. The aspirin responses were screened using several methods, in particular, the VerifyNow® Aspirin assay. Aspirin resistance was defined by at least two of the following three criteria: from a VerifyNow® assay result ≥550 aspirin reaction units, 0.5 mg/mL AA-induced aggregation ≥20%, and 10 µmol/L adenosine diphosphate (ADP)-induced aggregation ≥70%. Thirty patients (6.2%) were found to be aspirin resistant and were randomized to receive either low-dose aspirin plus omega-3 fatty acids (1 to 4 g/day) or aspirin 325 mg daily. The subjects were then retested after 30 days.

After treatment, twelve patients (80%) who received omega-3 fatty acids and 11 patients (73%) who received aspirin 325 mg daily were no longer aspirin resistant. Moreover, there was a significant reduction in AA- and ADP-induced aggregation and a lower VerifyNow® assay result. Additionally, plasma levels of TXB₂ were reduced in both treatment groups (Figure 2, page 42).9

More recently, Gajos et al demonstrated in an investigator-initiated, prospective, single-center, double-blind, placebo-controlled, randomized study that omega-3 polyunsaturated fatty acids compared with placebo...
Similarly, maximal platelet aggregation was consistently lower after 1 month of treatment with omega-3 polyunsaturated fatty acids compared with placebo.  

Conclusions

In summary, despite a remarkable body of literature focusing on so-called aspirin resistance, its definition, diagnosis, prevalence, causes, and clinical consequences are still uncertain, particularly when COX-1-specific platelet function assays are used to detect it. The previously reported ability of aspirin resistance, as detected by non-COX-1 specific assays, to predict poor cardiovascular outcomes may simply reflect high residual on-treatment platelet reactivity.

While splitting the dose of aspirin throughout the day in specific patient populations (eg, diabetic patients with high body mass indexes, patients with known inflammatory states) and adding omega-3 polyunsaturated fatty acids to aspirin regimens have been shown to overcome the AA-hyperresponsive platelet phenotype in COX-1 specific assays, it remains unclear if these strategies concomitantly improve outcomes. Hence, they cannot be recommended at the present time. Similarly, increasing the aspirin dose did not prove to be beneficial in lowering the number of hard cardiovascular events in the OASIS 7 trial, but did potentially increase the number of gastrointestinal bleeds. Altogether, the available data question the existence of a pure aspirin resistance phenomenon and provide a strong rationale for not modifying aspirin regimens unless it can be shown that this translates into a meaningful clinical improvement.

HOW TO OVERCOME CLOPIDOGREL RESISTANCE

Clopidogrel is an active prodrug that requires oxidation by multiple hepatic cytochrome P450 enzymes to generate an active metabolite, which selectively inhibits the P2Y12 receptor, resulting in an irreversible blockade for the life span of the platelet. The challenge is to overcome P2Y12 resistance. Remarkably, P2Y12 resistance is entirely due to the variable individual production of clopidogrel's active metabolite after ingestion; signal transduction at the level of the platelet receptor is well preserved in clopidogrel poor-responders.  

Increasing the dosage of clopidogrel

The first option investigated for overcoming P2Y12 resistance after clopidogrel ingestion was to increase the dose of clopidogrel. In the ALBION (Assessment of the best Loading dose of clopidogrel to Blunt platelet activation, Inflammation and Ongoing Necrosis) trial, 103 patients with non-ST-segment-elevation ACS were randomized to receive a 300, 600, or 900 mg loading dose of clopidogrel. Twenty-four hours after the loading dose, all patients were started on a regimen of clopidogrel 75 mg/day and aspirin 100 mg/day. The authors used aggregometry, flow cytometry, and measured inflammatory biomarkers and myonecrosis markers at all of the time points during the 24 hours after randomization, and patients underwent a follow-up at 30 days to record clinical outcomes and adverse events.

This trial demonstrated that loading doses higher than 300 mg were associated with a significantly faster onset of inhibition of platelet aggregation compared with the standard 300 mg clopidogrel loading dose. A significant dose-response was also observed for the VASP index, a measure of P2Y12 receptor inhibition, despite bleeding rates that were similar. Importantly, no significant differences in the degree of platelet aggregation or activation were noted between the 600 and 900 mg clopidogrel loading doses. Consistent findings were also provided by the ISAR CHOICE (Intracoronary Stenting and Antithrombolytic Inhibition Regimen Choice) study, which suggested that increasing the clopidogrel dose to 600 mg may improve platelet inhibition compared with the standard 300 mg loading dose.
botic Regimen: Choose between 3 High Oral doses for Immediate Clopidogrel Effect) trial, which showed incremental platelet inhibition after the 600 mg, but not the 900 mg, clopidogrel loading dose compared with the 300 mg dose. Von Beckerath et al enrolled 60 patients who were admitted to hospital for coronary angiography. They were randomized to one of three clopidogrel loading doses (300, 600, or 900 mg). The primary end point of the study was maximal ADP-induced platelet aggregation 4 hours after administration of clopidogrel. An increase in clopidogrel loading dose from 600 to 900 mg did not result in suppression of ADP-induced platelet aggregation, which was explained by a lack of further increase in the plasma level of clopidogrel’s active metabolite.15

Angiolillo et al explored the effect of modulating the maintenance dose of clopidogrel in the OPTIMUS (Optimizing anti-Platelet Therapy In diabetes Mellitus) study. They hypothesized that a daily maintenance dosage of 150 mg clopidogrel in high-risk patients (eg, diabetics and patients with a poor response to clopidogrel) would enhance platelet inhibition compared with the conventional dosage (75 mg) in such patients. After randomization, the assigned maintenance dosage regimen was maintained for 30 days, at which time platelet function was reassessed. This study demonstrated that patients assigned to the 150 mg dosage experienced a significant reduction in poor response. When all patients resumed the standard dosage of 75 mg clopidogrel daily, platelet function returned to baseline for poor-responders. However, one month after randomization, only 40% of patients in the high maintenance dose group had become full responders to clopidogrel (Aggmax after stimulus of 20 µmol/L ADP <50%).16

To further investigate the clinical implications of a higher clopidogrel loading and maintenance dose on hard clinical endpoints, CURRENT-OASIS 7 tested a strategy of doubling the clopidogrel loading dose (from 300 to 600 mg) and maintenance doses (from 75 to 150 mg daily for 7 days) in 25 087 ACS patients. In patients who underwent PCI, but not in the overall intention-to-treat population, the doubled clopidogrel dose significantly reduced the rate of myocardial infarction (MI) (12%) and stent thrombosis (29%). In the overall population, there was an increase in bleeding events. In particular, a current major bleeding increase of 25% (P=0.01) and a current severe bleeding increase of 23% (P=0.03) were observed (Figure 3).7

The GRAVITAS (Gauging Responsiveness with A VerifyNow® assay—Impact on Thrombosis And Safety) study is currently randomizing patients who show a poor response to a standard clopidogrel regimen (based on VerifyNow® P2Y12 reaction score >235 units) of 150 mg or 75 mg clopidogrel daily after successful PCI with drug-eluting stent (DES) implantation. The primary end point will be the time to first occurrence of cardiovascular death, nonfatal MI, or stent thrombosis, and platelet reactivity will be analyzed after 30 days and 6 months.17 This study will explore whether a selective strategy of doubling the maintenance clopidogrel dose in patients with high on-treatment platelet reactivity results in a decrease in ischemic events without an increase in bleeding complications.
Replacing clopidogrel with ticlopidine

An alternative for overcoming clopidogrel resistance is to administer ticlopidine (a first-generation thienopyridine). In a crossover study, Campo et al demonstrated that patients who were nonresponders to clopidogrel were largely responsive to ticlopidine and attained a higher overall level of platelet inhibition. The authors enrolled 568 patients who underwent coronary angiography. All patients with ST-segment-elevation MI received aspirin (250 mg intravenously), heparin (50 to 70 U/kg), and glycoprotein IIb/IIIa inhibitors. Patients with stable angina received aspirin (100 mg once a day) for at least 7 days and clopidogrel for at least 6 hours prior to the procedure. Aspirin (100 mg once a day) was provided to all of the patients indefinitely, whereas thienopyridines were administered for 1 or 6 months according to the implanted stent. Patients had outpatient visits every 4 months. The clinical end points were death, reinfarction, target vessel revascularization, or a major adverse cardiac event (MACE). Platelet aggregation measurement was performed at baseline before thienopyridine administration (clopidogrel 300 mg loading dose followed by 75 mg/day), at visit T1 (clopidogrel steady state, 5 to 7 days after baseline), and at visit T2 (ticlopidine steady state, 7 to 10 days after T1). The timing of T1 was selected on the basis of previous findings that suggested that the maximum inhibitory response to a standard clopidogrel regimen occurs within 24 hours and appears to last for 5 to 30 days. After T1, clopidogrel was substituted with ticlopidine (500 mg loading dose, followed by 250 mg twice daily). The timing of T2 allowed the clearance of clopidogrel and provided ticlopidine-induced platelet inhibition at steady state.

Clopidogrel and ticlopidine resistance were defined as: (i) an absolute difference between baseline and posttreatment Aggmax ≤10%, or (ii) platelet-aggregation inhibition <20%. This study demonstrated that responsiveness to both clopidogrel and ticlopidine was normally distributed, and platelet aggregation at T1 did not differ compared to T2. Thirty (21%) and 28 (19%) patients were clopidogrel and ticlopidine nonresponders, respectively. Only 5 patients (3.5%) were nonresponders to both clopidogrel and ticlopidine (class effect), whereas 25 patients (83%) who were clopidogrel nonresponders at T1 were responsive to ticlopidine; therefore, a higher level of platelet inhibition at T2 (platelet aggregation, 69±15 vs 44±18, P<0.01) (drug-specific response) was reached. In contrast, 23 patients who were responsive to clopidogrel demonstrated resistance to ticlopidine at T2 (platelet aggregation, 46±15 vs 70±15, P<0.01) (drug-specific response) (Figure 4).18

Adding a third antiplatelet agent

In patients that demonstrated a poor response to clopidogrel, the addition of a third antiplatelet agent was also tested. Clopidogrel poor-responders undergoing PCI are known to be at increased risk of periprocedural MI. A quick-acting antiplatelet agent may be required to rapidly overcome high on-treatment platelet reactivity in these patients and improve the outcomes.

We hypothesized that intensifying platelet inhibition with a tailored infusion of tirofiban, a platelet glycoprotein IIb/IIIa inhibitor, in aspirin and/or clopidogrel poor-responders, based upon a point-of-care assay, may reduce the incidence of MI after elective coronary angioplasty compared with standard care. Between February 2006 and June 2008, 1277 patients were screened and 263 patients were enrolled in the study. Periprocedural MI occurred in 27 (20.4%) and 46 (35.1%) patients in

![Graph showing percentage of nonresponders to clopidogrel and ticlopidine therapy assessed by two different methods, Aggmax and inhibition of platelet aggregation.](Image)

**Figure 4.** Percentage of nonresponders to clopidogrel and ticlopidine therapy assessed by two different methods, Aggmax and inhibition of platelet aggregation.

**Definition 1** is the absolute difference between baseline and posttreatment Aggmax ≤10%, while **definition 2** is based on an IPA <20%.

**Abbreviation:** IPA, inhibition of platelet aggregation.

**Modified from reference 18:** Campo et al. J Am Coll Cardiol. 2007;50:1132-1137. © 2007, American College of Cardiology.
the tiroliban and placebo groups, respectively (relative risk for tiroliban versus placebo, 0.58, 95% confidence interval [CI], 0.39 to 0.88; \( P=0.0089 \)) (Figure 2). At 30 days, the cumulative incidence of MACE was also reduced in the tiroliban group (10.7% vs 3.8%; \( P=0.031 \)). Thus, the addition of glycoprotein IIb/IIIa inhibitors at the time of PCI may represent an appealing strategy to immediately lower residual platelet activation, which has well known implications in terms of periprocedural ischemic events. Yet, as poor responsiveness to clopidogrel may harbor increased risk for recurrences well after PCI, a complementary long-term oral strategy would be highly desirable (Figure 5).\(^\text{19}\)

Another compound with potential as a third antiplatelet agent is cilostazol. Cilostazol is an inhibitor of phosphodiesterase 3 that increases intraplatelet cAMP levels. In patients undergoing coronary stenting, treatment with cilostazol in addition to aspirin and clopidogrel therapy (“triple antiplatelet therapy”) is associated with a reduced risk of stent thrombosis and MACEs. In particular, cilostazol has been shown to be effective in high-risk diabetic patients. In OPTIMUS 2, type 2 diabetes patients on dual antiplatelet therapy received either a 100 mg dose of cilostazol or placebo twice daily for 14 days and then crossed-over treatments for another 14 days. Platelet function was monitored at three time points: baseline, 14 days after randomization, and 14 days after treatment crossover. The P2Y\(_{12}\) reactivity index was significantly lower following cilostazol treatment compared with placebo, which suggests that the addition of this drug may overcome residual high platelet reactivity after clopidogrel ingestion.\(^\text{20}\)

Recently, a study demonstrated that triple antiplatelet therapy may produce more potent inhibition of platelet aggregation in diabetic patients undergoing coronary stent implantation. Tae-Hyun Yang et al enrolled 55 type 2 diabetes patients who had undergone DES implantation. Chronic antiplatelet therapy was stratified according to the status of the antiplatelet therapy. Platelet aggregation between dual (aspirin plus clopidogrel, n=34) and triple therapy (aspirin, clopidogrel, plus cilostazol) groups was compared using LTA. The two groups had similar clinical and procedural characteristics. In the triple therapy group, the maximal ADP-induced platelet aggregation was significantly lower than the dual therapy group, and there were no differences in diabetic treatment (oral hypoglycemic agent vs insulin) or diabetic control. In conclusion, triple antiplatelet therapy demonstrated more potent inhibition of maximal ADP-induced platelet aggregation in type 2 diabetes patients receiving chronic antiplatelet therapy. This evidence must be weighed against the fact that no properly powered study to assess the effect of cilostazol on clinical end points has so far been performed, and the use of cilostazol has been associated with potentially serious side effects in the small cohort studies completed.\(^\text{21}\)

Replacing clopidogrel with a newer P2Y\(_{12}\) receptor blocker

A more realistic option to overcome hyporesponsiveness to clopidogrel is the use of more potent, more consistent oral P2Y\(_{12}\) receptor blockers, including prasugrel and ticagrelor. Both drugs have previously been tested in phase 3 studies of large randomized controlled trials against clopidogrel, and both drugs were associated with improved outcomes. Prasugrel is a third-generation thienopyridine. This drug offers more complete platelet inhibition through more efficient drug metabolism (with nearly 90% transformation into the active metabolite).

The drug was first tested in phase 2 of the JUMBO-TIMI (Joint Utilization of Medications to Block platelets Optimally–Thrombolysis In Myocardial Infarction) 26 trial, whose results suggested improved efficacy without an increase in bleeding complications.\(^\text{22}\) Recently, the results of TRITON-TIMI (TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibitionN with prasugrel–Thrombolysis In Myocardial Infarction) 38, a phase 3 study, demonstrated that treatment with prasugrel produced a significantly lower rate of the combined primary outcome of cardiac death, nonfatal MI, and stroke than treatment with clopidogrel. A total of 13 608 patients with ACS undergoing PCI were randomized to prasugrel or clopidogrel. The trial demonstrated benefits in the pra-
sugrel group, including reduced MI, stroke, and cardiovascular death. Prasugrel significantly reduced urgent revascularization, MI, and stent thrombosis irrespective of glycoprotein IIb/IIIa use. Importantly, there was no effect of treatment on mortality or stroke rates. However, prasugrel increased the risk of major bleeding complications, including life-threatening and fatal bleeding.

A post hoc analysis demonstrated that there are three patient subgroups that failed to derive any clear benefit from treatment: namely those with a previous transient ischemic attack or stroke, patients >75 years old; and those that weigh <60 kg. In contrast, sound clinical benefits have been shown in ST-segment–elevation MI and diabetic patients. Prasugrel is not affected by the presence of loss-of-function 2C19*2 alleles. Yet, a direct comparison of prasugrel versus clopidogrel in this high-risk patient population is lacking.

A more appealing balance between efficacy and safety has been shown by the use of ticagrelor. Ticagrelor is an orally active P2Y₁₂ inhibitor that does not require hepatic activation, providing a more rapid and complete antiplatelet effect than clopidogrel. The clinical efficacy and safety of ticagrelor was tested in the PLATO (PLATelet inhibition and patient Outcomes) trial. In this multicenter, double-blind, randomized trial, ticagrelor was compared to clopidogrel for the prevention of cardiovascular events in 18,624 patients admitted to hospital with ACS, with or without ST-segment–elevation. The authors found that after 12 months, the primary end point (a composite of death from vascular causes, MI, and stroke) occurred in 9.8% of patients that received ticagrelor compared with 11.7% of those that received clopidogrel. Remarkably, the rates of all-cause and cardiovascular death were also significantly reduced with ticagrelor. No significant difference in the rates of major bleeding was found between the ticagrelor and clopidogrel groups. This may largely reflect the reversible nature of P2Y₁₂ inhibition (Figure 6). Elinogrel is a new oral and intravenous P2Y₁₂ receptor blocker. It is a direct-acting, reversible P2Y₁₂ receptor antagonist. The ERASE MI (Early Rapid reversAl of platelet thromboSis with intravenous Elinogrel before PCI to optimize reperfusion in acute Myocardial Infarction) trial is a pilot phase 2a randomized, double-blind, placebo-controlled, dose-escalation study to evaluate the safety and tolerability of escalating doses of elinogrel. The incidence of bleeding events was infrequent and appeared to be similar in patients treated with all doses of elinogrel versus placebo. This drug has also recently been investigated in a large phase 2 study, the INNOVATE PCI (INtraveNous and Oral administration of elinogrel, a selective and reversible P2Y₁₂-receptor inhibitor, versus clopidogrel to eVAluate Tolerability and Efficacy in nonurgent Percutaneous Coronary Interventions patients) trial, and the results of this study are soon to be presented. In conclusion, there has been tremendous progress in our understanding of the clinical implications of high on-clopidogrel residual platelet reactivity, which has been paralleled by the development of new strategies to overcome so-called clopidogrel resistance. However, clinical evidence favoring the use of these new pharmacological options versus standard options is currently still limited. While results of the ongoing GRAVITAS may shed light on the value of doubling the maintenance dose of clopidogrel selectively in clopidogrel poor-responders, the use of prasugrel today and ticagrelor tomorrow (pending European Medicines Agency and Food and Drug Administration approval) appears an attractive and clinically sound strategy.

Conclusions

In conclusion, there has been an increasing awareness of the clinical implications of high on-clopidogrel residual platelet reactivity, which has been paralleled by the development of new strategies to overcome so-called clopidogrel resistance. However, clinical evidence favoring the use of these new pharmacological options versus standard options is currently still limited. While results of the ongoing GRAVITAS may shed light on the value of doubling the maintenance dose of clopidogrel selectively in clopidogrel poor-responders, the use of prasugrel today and ticagrelor tomorrow (pending European Medicines Agency and Food and Drug Administration approval) appears an attractive and clinically sound strategy.
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In the second half of the nineteenth century, it was concluded from careful pathological studies that platelet thrombi were formed when a blood vessel was injured. Virchow provided a careful analysis of thrombus resolution and the appearance of colorless blood corpuscles (platelets) as the thrombus disintegrates. The etiology of arteriosclerosis was a source of great controversy in the mid-nineteenth century, and it was the pathologist Karl Rokitanski who proposed that arteriosclerosis was initiated by clots adhering to arteries, which subsequently evolved into arteriosclerotic plaques. Rokitanski, who worked in Vienna as an imaginative pathologist, tried to explain all diseases in terms of “crosis” of the blood: he believed that any disease resulted in a chemical disorder of blood, and he hypothesized that most systemic diseases could be attributed to a specific problem in the blood. This hypothesis was strongly refuted by Virchow.

Research in platelet function in health and disease rapidly expanded from the 1950s onwards, facilitated by advances in experimental technologies that permitted studies on the role of platelets in hemostasis. Baumgartner developed a methodology for evaluating the microscopic interaction of platelets on arterial endothelial cells. A complementary in vivo experimental preparation to study the role of platelets in hemostasis was the hamster cheek pouch model. Probably the most notable technical advance was the application of the aggregometer to the study of quantitative aspects of platelet aggregation by Born. Born, who had worked as a doctor in the British Army in World War II, had observed how the Hiroshima atomic bomb had caused multisystem bleeding, due to thrombocytopenia secondary to marrow depression. Born subsequently studied for a PhD in the Department of Pharmacology in Oxford (1956) and selected platelets as an isolated tissue in order to study the vascular effects of their contents, particularly 5-hydroxytryptamine. Subsequent studies by him showed that platelets had excess adenosine triphosphate (ATP), which was rapidly broken down in clotting blood. At the same time, another group showed that adenosine diphosphate (ADP) caused platelet aggregation. This led to the hypothesis that ADP was formed from ATP released from platelets, and that ADP causes platelet aggregation in both hemostasis and thrombosis. In order to study this mechanism, he developed the optical aggregometer (Figure 1), which allows platelet aggregation to be measured quantitatively via the detection of aggregation-induced optical changes.

It had been known since the 1880s that platelets played a central role in the formation of either a hemostatic plug or a mural thrombus. But details of how this came about only became known with the application of the technical advances from 1955 onwards. As a consequence of these advances, there was a great upsurge in studies on platelet function. Amongst the nu-

**Selected Abbreviations and Acronyms**

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<th>Abbreviation</th>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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merous scientific groups studying the function of platelets between 1960 and 1980, certain ones stand out. In 1960, Professor Born was appointed to the Royal College of Surgeons and was joined by John Vane as senior lecturer. This partnership, the Thrombosis Research Group, lasted 13 years, terminating in 1973. Salvador Moncada joined them for two years, in 1971, and all three made notable contributions to the understanding of the pathophysiology of platelet function.

In the same period, Fraser Mustard, working in the Department of Pathology at the University of Toronto, and subsequently at McMaster University in Hamilton, led a successful research group, which included Jack Hirsch, Sean Moore, and Miriam Packham, who remained in Toronto. Other notable contributors to the platelet story include Harvey Weiss in New York, Baumgartner in Roche (Basel), and Verstraete in Liege (Belgium). They and many others helped describe the complex and subtle function of platelets, which made the identification of acceptable modulators of their function a major and prolonged scientific challenge.

Platelets play a key role both in initiating normal hemostasis and arterial thrombosis. The key elements are illustrated in Figure 2. Whilst the association between platelets and thrombus formation was first described between 1842 and 1874, it was not until McFarlane’s proposal that blood coagulation depends upon a sequential series of clotting factors, whose activation takes place on the surface of the platelet as it adheres to an injured vessel wall, that the current model of hemostasis evolved. Exposure of platelets to an injured vessel results in a triple-phased sequence of aggregation, adhesion, and secretion. The details of the biochemical mechanisms mediating these changes are the subject of numerous reviews.

FIRST-GENERATION PLATELET INHIBITORS

Cyclooxygenase inhibitors

Aspirin

Aspirin (acetylsalicylic acid) was first marketed by the Bayer company in 1899 as an analgesic and antipyretic agent, administered at doses of 0.5-1 g two to four times daily. Its effects in increasing bleeding time had been known for many years, as was the difference between the short duration of action as an analgesic compared with its prolonged duration of action on bleeding time. The discovery of prostaglandins (PGs) by von Euler between 1936 and 1960, together with subsequent studies by Vane and colleagues at the Royal College of Surgeons between 1969 and 1972, showed that aspirin irreversibly blocked the formation of the proaggregatory PG thromboxane A2 (TXA2) by the cyclooxygenase (COX) enzyme in platelets.

Other papers describe in detail different aspects of the mode of action of aspirin, which will not be discussed further in this review. Suffice to say that the meta-analysis by the Antiplatelet
Trialists’ Collaboration demonstrated a 25% relative risk reduction in vascular death, myocardial infarction, and stroke predominantly associated with aspirin treatment in comparison with placebo.\(^1\) It is now the gold standard for the secondary prevention of cardiovascular disease, and also for the treatment of acute ischemic syndromes.

Platelets, like the majority of cells, contain PG isoenzyme H-synthase, or COX-1 as it is better known. This enzyme converts arachidonic acid to both prostaglandin \(G_2\) (PGG\(_2\)) and prostaglandin \(H_2\) (PGH\(_2\)), and PGH\(_2\) is subsequently converted to TXA\(_2\), which is a singularly potent stimulant of irreversible platelet aggregation and release. COX-1 inhibitors reduce TXA\(_2\)-induced platelet aggregation, resulting in prolongation of bleeding time. It is probable that the irreversible selective acetylation of serine\(^5\) distinguishes aspirin from the reversible inhibitor sulfinpyrazone, which has relatively weak COX-1 inhibitory activity. An alternative COX-1 inhibitor is indobufen (200 mg bid), a potent COX inhibitor that inhibits TXA\(_2\) formation as effectively as aspirin. Consequently, it has beneficial effects in reducing coronary artery graft occlusion, but its effectiveness in other vascular diseases is unproven.\(^2\)

### Adenosine and platelet function

One of the critical observations made by Born, using his novel aggregometer, was the demonstration that ADP, which is found in normal platelet granules, is a potent stimulant of platelet aggregation.\(^10\) He showed that this action was specific to ADP and not shared by related nucleotides. Mustard’s group, at the same time, showed that ADP-induced aggregation of platelets caused experimental infarction in swine, providing a key link between platelet activation and tissue ischemia.\(^22\)

### Dipyridamole

Dipyridamole was discovered by Boehringer Ingelheim in the 1950s and was shown to have vasodilatory effects. Since nitrate-based vasodilating compounds were already in use to treat angina pectoris, dipyridamole was introduced clinically for this indication in the early 1960s as a long-acting vasodilator. At the same time, Born was studying 2 substituted analogues of adenosine as potential inhibitors of the platelet aggregating actions of ADP, based on his hypothesis that “it is conceivable that AMP or some other substance could be used to inhibit or reverse platelet aggregation in thrombosis.”\(^7\) The relationship between relaxation of vascular smooth muscle and platelet function was based on the well-known cyclic adenosine monophosphate (AMP) mechanism. Dipyridamole inhibits the phosphodiesterase enzyme, which degrades cyclic AMP to 5-AMP. As a consequence of the increase in intraplatelet cyclic AMP, platelet aggregation is irreversibly inhibited. This elegant hypothesis has been challenged because there are doubts that the dose of dipyridamole used clinically for platelet inhibition provides an adequate free drug concentration. Thus, this proposed mode of action of dipyridamole is not supported by data from extensive clinical studies. Alternative modes of action proposed include inhibition of eicosanoids due to changes in concentration of prostacyclin and prostaglandin \(D_2\) (PGD\(_2\)), and improvement of impaired platelet turnover in patients with atherosclerotic vascular disease.\(^23\) Extensive clinical trials in secondary prevention of acute myocardial infarction, transient ischemic attacks, and coronary graft patency—either as monotherapy in the dose range 75-400 mg daily or coadministered with aspirin—have still not convinced critics as to its proven utility.\(^24\) Nevertheless, this serendipitously discovered drug is still widely prescribed for cardiovascular indications.

### Sulfinpyrazone (Anturan)

The potential utility of sulfinpyrazone as an antiplatelet agent originated from a fortuitous observation by Mustard’s group at the University of Toronto in the early 1960s.\(^25\) They demonstrated that patients with gout had shortened platelet survival and platelet turnover due to excess production of uric acid, accompanied by increased early coagulation activity. They attributed the increase in vascular disease in patients with gout to these platelet abnormalities. The group then studied the effects of sulfinpyrazone (400 mg daily for five weeks), marketed by the Geigy company as a uricosuric agent, on platelet function in subjects with gout. This course of treatment prolonged platelet survival and reduced platelet turnover in these subjects, with no change in blood coagulation.\(^26\)

These observations led to pilot clinical studies that showed that sulfinpyrazone lengthened platelet survival and decreased platelet turnover in patients with prosthetic mitral valve implants,\(^27\) recurrent venous thrombosis, and coronary artery disease.\(^28\) As a consequence, a large multicenter trial was initiated in 1975, the Anturan Reinfarction Trial. It was designed to compare the effects of sulfinpyrazone and placebo on the rates of cardiac mortality in patients with recent myocardial infarction. Initially these patients were followed for about nine months, but subsequently results were reported for a follow-up of sixteen months. There were 106 cardiac deaths, with a 32% reduction in cardiac mortality and a 43% reduction in sudden death.\(^29\)\(^30\)

The design and analysis of this trial were heavily criticized by the US Food and Drug Administration. Although the Italian study group of the Anturan Reinfarction Trial did demonstrate a reduction in combined fatal and non-fatal myocardial infarction treated over nineteen months, sulfinpyrazone...
has never become a widely used anti-platelet agent because of the controversy over the design and execution of the trial. Perhaps one of the contributing factors is that sulfinpyrazone is only a weak cyclooxygenase inhibitor, i.e., 60%, whereas aspirin causes irreversible inhibition of this enzyme.

**Clofibrate**

Clofibrate was discovered in 1960 as a novel hypolipidemic agent.\(^\text{31}\) By chance, it was observed that clofibrate and a related analogue, halofenate, inhibited collagen-induced platelet aggregation, prolonged platelet survival, and inhibited the second phase of ADP-mediated aggregation. These positive, unanticipated effects on platelet function led the World Health Organization to conduct a trial on clofibrate.\(^\text{32}\) This was performed in asymptomatic subjects with elevated cholesterol, and resulted in a 20% reduction in the incidence of myocardial infarction. But there was an unexplained increase in deaths from unrelated causes, the reason for which has never been explained.\(^\text{33}\) In addition, the well-designed Coronary Drug Research Group Trial failed to show a reduction in cardiac-related morbidity and mortality. It seems reasonable to conclude that the observed effects on platelet function were not sufficient to be of therapeutic benefit.

**Hydroxychloroquine sulfate**

In 1960, Madow\(^\text{34}\) showed that patients with peripheral arterial disease treated with hydroxychloroquine had reduced aggregation of erythrocytes. The rationale for this study was based on previous experiments in malaria-infected monkeys that showed that this compound “unplugged” vessels locked by aggregates of erythrocytes. Rosenberg showed that it also reduced experimental thrombosis in rabbits,\(^\text{35}\) while other investigators showed that it also reduced platelet aggregation. An extensive single-centre study investigating hydroxychloroquine sulfate (600-800 mg daily) was performed by Chamley in Wrightington Hospital.

<table>
<thead>
<tr>
<th>Class</th>
<th>Mode of action</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. P2Y(_{12}) inhibitors</strong></td>
<td>Reversible blockade of P2Y(_{12}) platelet receptor</td>
<td>In comparison with clopidogrel, greater and faster onset and offset of inhibition of receptor, mode rapid effect.(^\text{37})</td>
</tr>
<tr>
<td>1.1 Ticagrelor AZD6140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 Cangrelor</td>
<td>Short-acting parenteral inhibitor</td>
<td>Efficacious in PTC therapy as clopidogrel</td>
</tr>
<tr>
<td>1.3 Elinogrel</td>
<td>Oral/parenteral P2Y(_{12}) inhibitor</td>
<td>In phase 2 trials—ClinicalTrials.gov (NCT00751231)</td>
</tr>
<tr>
<td><strong>2. Direct thrombin receptor antagonists</strong></td>
<td>Blocks protease receptor (PAR-1/4) on platelet</td>
<td></td>
</tr>
<tr>
<td>2.1 Vorapaxar (SCS530348)</td>
<td>Blocks alpha-thrombus chain action</td>
<td>Suggested that it reduces ischemic events without increased bleeding</td>
</tr>
<tr>
<td>2.2 E555</td>
<td>Blocks tethered ligand binding site</td>
<td></td>
</tr>
<tr>
<td><strong>3. Glycoprotein IIb/IIIa receptor inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.1 Abciximab</td>
<td>These receptors on adherent platelets are activated by fibrinogen and von Willebrand factor</td>
<td>These are final common activation pathway for all endogenous ligands(^\text{38})</td>
</tr>
<tr>
<td>3.2 Tirofiban</td>
<td>Small nonpeptidic tyrosine derivative with more rapid offset of action</td>
<td></td>
</tr>
<tr>
<td>3.3 Eptifbatide</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>4. PDE inhibitors</strong></td>
<td>Inhibits cAMP breakdown</td>
<td>Probably several other actions; originally a coronary vasodilator</td>
</tr>
<tr>
<td>4.1 Dipyridamole</td>
<td>Selective inhibitor of PDE-3 increases tissue cAMP; antiplatelet/vasodilator</td>
<td>Limited efficacy in POAD</td>
</tr>
<tr>
<td>4.2 Cilostazol</td>
<td></td>
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</tbody>
</table>

**Table 1. Alternative mechanisms of platelet inhibition.**

Abbreviations: cAMP, cyclic adenosine monophosphate; PAR, protease-activated receptor; PDE, phosphodiesterase; POAD, peripheral occlusive arterial disease; PTC, plasma thromboplastin component.
near Manchester between 1974 and 1976 as a prophylactic treatment for the development of deep vein thrombosis and pulmonary embolism associated with orthopedic surgery. The incidence of pulmonary embolism fell from a historical 2.3% between 1962 and 1971 to 0.1% between 1974 and 1976. Charnley declined a trial versus placebo, and perhaps it was for this reason that this treatment was not pursued elsewhere. This summary of first-generation antiplatelet agents could have included a wide variety of other chemical agents that have been shown to modulate platelet function, mainly in in vitro experiments. These were summarized in a comprehensive review by Mustard and Packham in 1970, but none were taken forward for clinical evaluation.11

SUBSEQUENT DISCOVERIES OF NOVEL ANTIPLATELET DRUGS (Table I)37,38

Irreversible P2Y12 antagonists

The most important discovery of antiplatelet drugs, apart from that of aspirin, is the thienopyridines, a group of drugs comprising the first-in-class ticlopidine, and clopidogrel and prasugrel. The discovery of this class of novel antiplatelet agents started with the discovery of tinoridine by the Japanese pharmaceutical company Yoshi-tomi in 1972. Their objective was to design an improved nonsteroidal anti-inflammatory agent whose mode of action was to inhibit OH free radical generation. Research scientists at Sanofi in France, led by Dr J-P Maffrand, synthesized tinoridine analogues, one of which was ticlopidine (Figure 3). This compound underwent general in vivo preclinical pharmacological evaluation. Ironically, it had no anti-inflammatory activity, but it did inhibit ADP-induced platelet activation in ex vivo tests on rat platelets.10 This provides yet another example of the pivotal role of serendipity in novel drug discovery. Even more remarkable is the fact that ticlopidine does not inhibit ADP platelet aggregation in vitro tests, but only in vivo. Thus, ticlopidine would not have been identified as an antiplatelet agent using modern, high-throughput in vitro screening techniques (Table II). Another facet of the role of serendipity in drug discovery is the proposal made by Burnstock in 1971 that ATP is a neurotransmitter in nonadrenergic, noncholinergic nerves. This was followed by his hypothesis that cells have purinergic receptors activated by ATP/ADP. The relevance of this to the discovery of ticlopidine is that subsequent research showed that metabolites of ticlopidine irreversibly block purinergic receptors on platelets. This was a mode of action unrelated to that of aspirin-induced inhibition of TXA2 stimulation of platelet aggregation. Subsequent studies on purinergic-mediated platelet effects have shown that the ADP receptor is a G-protein–coupled receptor, which is now subclassified as purinergic P2Y1 and P2Y12 receptors.46

Ticlopidine was extensively evaluated in cardiovascular diseases and was as effective as aspirin in reducing vascular thrombotic events. However, it caused increases in plasma cholesterol, neutropenia, and, very rarely, thrombotic thrombocytopenic purpura. Substitution of a carboxymethyl group on the ticlopidine structure produces clopidogrel, which has been shown to be a very effective and well-tolerated inhibitor of platelet aggregation. In 2000, scientists at the Japanese pharmaceutical firm Sankyo described the discovery of compound CS-747, now subclassified as purinergic P2Y12 receptors.47

Table II. Serendipitous aspects of the discovery of ticlopidine.
Abbreviation: NSAID, nonsteroidal anti-inflammatory drug.

- The initial therapeutic target was tinoridine: an NSAID with antiperoxidative activity
- Ticlopidine, one of a series of thienopyridines, was screened for anti-inflammatory activity
- No anti-inflammatory action was detected, but general preclinical pharmacology in rats (in vivo) showed sustained inhibition of platelet function by an active metabolite (Figure 4, page 53)
- Purinergic receptors described by Burnstock

![Figure 3. Thienopyridine structures. Modified from reference 40: Lüs et al. Semin Thromb Hemost. 1999; 25:29-32. © 1999, Thieme Medical Publishers.](Image 248x534 to 466x693)
subsequently marketed as prasugrel in collaboration with the US pharmaceutical firm Lilly. Like ticlopidine and clopidogrel, prasugrel is a prodrug discovered by in vivo studies in rats. Its active metabolite, R138727, is an irreversible inhibitor of the platelet P2Y12 receptor. Its claimed advantage over clopidogrel is that plasma esterases convert the prodrug compound to a metabolite that requires only a single hepatic P450 (CYP3A) isoenzyme to form the active receptor inhibitor. In contrast, not only is clopidogrel inactivated by plasma esterases, but it also requires two P450 isoenzymes for activation, one of which (CYP2C19) can have genetically-determined loss-of-function polymorphisms that reduce its effectiveness in subjects with this polymorphism (Figures 3 and 4).40,42

Currently, clinicians have the choice of using either clopidogrel or prasugrel for the management of thrombotic vascular disease, though aspirin remains the gold standard for prophylactic use in arterial diseases. Since aspirin and the thienopyridines have clearly different modes of action for inhibiting platelet activation, ie, irreversible cyclooxygenase inhibition by aspirin versus irreversible purinergic receptor blockade by thienopyridines, dual therapy is a rational approach. Numerous trials are currently in progress to provide evidence-based data to guide their future application.48

Reversible P2Y12 antagonists

An alternative approach to inhibiting platelet aggregation induced by endogenous ADP via activation of the P2Y12 receptor is to induce reversible, rather than irreversible, receptor inhibition. The objective is to identify agents that have both a faster onset and offset of receptor inhibition, as well as to avoid the need for metabolic activation associated with the thienopyridines. There are three reversible inhibitors in phase 2/3 clinical trials. Ticagrelor (AZD6140), the most advanced compound, has been compared with clopidogrel in a large clinical trial in acute coronary syndromes.47 It has recently been approved by the European Medicines Agency for treatment of acute coronary syndromes based on the 18 000-patient PLATO (PLAtelet inhibition and patient Outcomes) trial.

Two other reversible inhibitors are cangrelor, which is only active when administered parenterally, and elinogrel, which is active whether administered orally or parenterally.50,51 The comparative efficacy/safety profiles of these compounds compared with either clopidogrel or prasugrel have yet to be established.

It must be emphasized that there are other important pathways involved in platelet activation and aggregation other than the P2Y12 receptor. The platelet has between 500 and 1000 ADP binding sites, of which about one third...
are P2Y1 receptors, while the endogenous platelet agonists thrombin and TXA2 have twice this number of receptors on platelets. There is, in addition, experimental evidence to suggest that the P2Y1 receptor is involved in shear-mediated platelet activation, which can be inhibited by the selective P2Y1 receptor blocker MF449 in experimental thrombosis.

The complex interplay between coagulation factors and factors released from activated platelets has resulted in the development of selective inhibitors of the interaction between platelets and coagulation factors, including the glycoprotein IIb/IIIa and thrombin receptors, which are also involved in platelet activation.

**Platelet glycoprotein receptor inhibitors**

The final common pathway for platelet activation is the functionally active glycoprotein IIb/IIIa on the surface of the platelet. Barry Coller’s group at the Mount Sinai School of Medicine in New York initiated studies to find selective inhibitors of the receptor in the early 1980s. They developed a selective murine monoclonal antibody and showed that there are between 40 000 and 80 000 glycoprotein IIb/IIIa receptors on the surface of platelets, spaced about 200 nm apart. These receptors are activated by selected sites on the fibrinogen molecule and also by von Willebrand factor, both of which lead to platelet recruitment on the injured endothelial surface. Furthermore, ADP, serotonin, TXA2, and thrombin can also activate these receptors.

In regard to selectivity of action, it is notable that the only other cell with similar glycoprotein IIb/IIIa receptors is the megakaryocyte. The fact that IIb/IIIa antagonists can block a range of endogenous platelet activating ligands results in their being more effective than either aspirin or the thienopyridines. A rare genetic condition causing the failure of expression of glycoprotein IIb/IIIa receptors on the platelet, Glanzmann thrombasthena, is associated with a tendency to bleed.

**Abciximab**

The first IIb/IIIa antagonist developed for clinical use in acute myocardial infarction patients undergoing reperfusion and stent placement was abciximab, made by Centocor and distributed by Lilly. This is a mouse/human chimeric monoclonal antibody 7E3Fab. It evolved from a series of studies on murine monoclonal antibodies performed by Coller’s group in the early 1980s. The antibody binds to the arginine-glycine-aspartate motif in the receptor. When administered parenterally (0.2 µg/kg bolus plus 10 µg/min for 12 hours), it significantly reduces ischemic events in infarct patients treated with aspirin and clopidogrel undergoing percutaneous coronary angioplasty. Despite their greater efficacy in inhibiting platelet function, the three glycoprotein IIb/IIIa inhibitors have not been shown to be more effective in clinical trials in arterial thrombosis, but have unfortunately been accompanied by a greater risk of bleeding.

**COMMENTARY**

Prevention and/or treatment of vascular thrombosis remains a large relatively unmet need. There is a fundamental yin-yang between preservation of physiological hemostasis and prevention of thrombus formation, triggered mainly by atherosclerotic lesions. The possibly unattainable target of dealing with thrombus formation using selective modulators of the endothelium-platelet-coagulation cascade remains a formidable target. There can be no doubt that there have been notable improvements in treating arteriovenous thrombosis over the last fifty years, in which modulators of platelet function have played a major role. The application of molecular biology to the analysis of platelet function has led to an unanticipated expansion in the elucidation of the nature of processes involved in platelet function. As a consequence, several new targets, whose modulation might lead to the discovery of more potent specific compounds, have been described. Whilst new discoveries are scientifically exciting, it is necessary to respect the complexity of the systems involved in platelet activation when designing selective modulators.

For example, once the central role of TXA2 in modulating platelet adherence and release was known, novel inhibitors of TXA2 synthase and TXA2 receptors were evaluated in clinical studies. Whilst they were clearly efficacious in preclinical models of thrombosis, they did not show improved efficacy in clinical trials in acute coronary syndromes. Thus, in considering the potential of exciting biological targets for the future, it may be prudent to accept that specific, selected modulators of platelet function may not necessarily improve treatment in comparison with established therapy. Furthermore, selective modulators of the coagulation cascade, eg, factor Xa inhibitors, might prove to be more efficacious and safer than the newer antiplatelet compounds. However, a recent study of the effects of factor Xa inhibitors in acute coronary syndrome was associated with a dose-dependent increase in bleeding events. Such observations emphasize the critical issue of both dose and concomitant therapy in different thrombotic syndromes, and place a considerable responsibility on those prescribing these novel agents.

In conclusion, the trail of discovery of effective antiplatelet agents is a protracted story, illustrating yet again the roles of clinical science, pathological investigation, and pharmacology in the translation of such observations into therapeutic advances.
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Richard Bing, born in Nuremberg, Germany, in 1909, was highly talented in so many ways, weighing up careers in medicine versus music. He made his mark in both, but shone in cardiology research. In 1947, when working at the Johns Hopkins Institute, he discovered that coronary sinus catheterization could define cardiac substrate metabolism. In 1961, I learnt of his work and concepts, when my urge was to become a cardiology researcher. What was the best route towards that ideal? Professor Andries Brink, a leading South African cardiologist, said: “Study cardiac metabolism.” “What’s that?” I asked in ignorance. “Go and read Richard Bing’s four articles in the American Journal of Medicine in 1953-4 and you will find out.”

So the bug bit me, thereby deciding that my major career interest would lie in the biochemistry and metabolism of the heart. But I was only one of many stimulated by Richard Bing’s pioneering discoveries that the human heart muscle was so metabolically active, which in turn created such widespread ripples. European scientists working on heart metabolism initiated the European Study Group in 1967. Meanwhile, his musical talent was also coming to the fore. In 1976, he invited me to the premier performance of his Requiem Mass, which alas was in far-off Pasadena. In 1978, when the Study Group became Research in Heart Metabolism under Pierre Hatt in France and Albrecht Fleckenstein in Germany, who were soon joined by Peter Harris in London. A similar awakening and growth of the Study Group in North America was led by George Rona, Robert Jennings, Narajan Dhalla, and others. One of the founders of the Study Group was Eors Bajusz, a strong believer in the notion of a new journal focused on heart muscle and its metabolism, which he asked me to edit. But, I said, “I can’t do it on my own. Richard Bing is the leader in the field, and we must have him on board.” Richard graciously agreed and became Senior Coeditor. Thus the Journal of Molecular & Cellular Cardiology was born, with its first issue in 1970. With Bing as Senior Coeditor and Sir Hans Krebs coming in later as Chief Consulting Editor (both escapees from Nazi Germany), articles flowed in and the journal flourished. Bing saw the big picture, I saw the detail, and the combination worked.

Looking at the first volume in 1970, Bing’s visionary leadership and my contacts via the Study Group led to a galaxy of outstanding papers by leaders in cardiac metabolism, including the following (listed in order of appearance of their articles): Albert Wollenberger, George Burch, Victor Ferrans, Wolfgang Kübler, Arnold Schwartz (Editor for Short Communications), Amanda Lochner, Hans Selze, Peter Harris, Pierre Hatt, Joseph Moravec, Bernard Swynghedauw, Winifred Nayler, Sigmundur Gudbjarnason, Robert Jennings, Marianne Legato, Felix Meerson, Lars Carlson, and several others. The first issue of 1971 contained the obituary of Dr W. Raab, one of the prime movers of the Study Group.

Bing brought together the somewhat different European and American sections of the Study Groups, having lived on both continents, as President of the International Study Group for Research in Cardiac Metabolism, from 1967 to 1973. Meanwhile, his musical talent was also coming to the fore. In 1976, he invited me to the premier performance of his Requiem Mass, which alas was in far-off Pasadena. In 1978, when the Study Group became

In Memoriam

Richard Bing
Scientist, Cardiologist, Musician, Editor, and Novelist

Lionel Opie, MD, DPhil, DSc, FRCP
Professor Emeritus of the Hatter Institute for Cardiovascular Research
at the University of Cape Town Medical School - Cape Town - SOUTH AFRICA

Richard Bing (1909-2010).

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Looking at the first volume in 1970, Bing’s visionary leadership and my contacts via the Study Group led to a galaxy of outstanding papers by leaders in cardiac metabolism, including the following (listed in order of appearance of their articles): Albert Wollenberger, George Burch, Victor Ferrans, Wolfgang Kübler, Arnold Schwartz (Editor for Short Communications), Amanda Lochner, Hans Selze, Peter Harris, Pierre Hatt, Joseph Moravec, Bernard Swynghedauw, Winifred Nayler, Sigmundur Gudbjarnason, Robert Jennings, Marianne Legato, Felix Meerson, Lars Carlson, and several others. The first issue of 1971 contained the obituary of Dr W. Raab, one of the prime movers of the Study Group.

Bing brought together the somewhat different European and American sections of the Study Groups, having lived on both continents, as President of the International Study Group for Research in Cardiac Metabolism, from 1967 to 1973. Meanwhile, his musical talent was also coming to the fore. In 1976, he invited me to the premier performance of his Requiem Mass, which alas was in far-off Pasadena. In 1978, when the Study Group became...
the International Society for Heart Research at a meeting in New Delhi, he played a recording of part of his Requiem Mass to the general acclaim of all. Then, to relax, he went with me to the Taj Mahal. Again he saw the big picture as he sat back and just looked and looked, and put himself into the history of that time. He wondered what type of music was played at the inauguration of the Taj and who spoke what language. 1978 was also the year of his last academic article on heart metabolism, on “Alcoholic heart disease and myocardial failure,” published in *Circulation*.

Thereafter we kept contact for many more years, first by letter and then by e-mail. More recently, and in his 90s, he appeared to value my opinion...
on his novelettes as they appeared. *Fifteen Lives and the Cat’s Story* was sent to me in 2004 with the inscription: “To my friend Lionel.” These stories, each in a different way, bore a relationship to Bing’s progression as a physician and scientist, and his avocation as a composer of music. But this was his third career, now as a writer, successfully embarked on in his late 80s and 90s. For many years he wrote a column entitled *Past Truth and Present Poetry* for the *Heart News and Views – The News Bulletin of the International Society for Heart Research*. In 2006, with Richard’s kind permission and that of Tom Ruigrok for *Heart News and Views*, and Nikki Bramhill for TFM Publishing Ltd, *Dialogues in Cardiovascular Medicine*, edited by Roberto Ferrari and David Hearse, started republishing 12 of Richard’s *Past Truth and Present Poetry* pieces, with lavish illustrations.

Upon receiving *Dialogues*, every time, with exquisite old-world graciousness, Richard would take the time to write—a real letter, with envelope and stamp, not an e-mail—to express his appreciation of the layout: he was 100 when he sent his last one: “I’m proud to have been included. You’re doing a wonderful job.” In his “Finale” in 2010 at the age of 101, he summarized the achievements of his life. “Foremost there were the pleasures of living, of seeing the world’s beauty, its lakes, mountains, stars, laughter and tears.” What an all-rounder he has been.

But that was typical of Richard, always creative, whether in metabolism of the heart or choral music or symphonies or novels, thus being by far the most multicreative person I have had the privilege of knowing and working with.
Aspirin Resistance

Summaries of Ten Seminal Papers

Chantal Pharand, PharmD

Selection of seminal papers by Chantal Pharand, PharmD
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Dialogues Cardiovasc Med. 2011;16:61-71

1. Aggregation of blood platelets by adenosine diphosphate and its reversal

2. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects

3. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man

4. Development of aspirin resistance in persons with previous ischemic stroke
   C. M. Helgason and others. Stroke. 1994

5. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death...
   J. W. Eikelboom and others. Circulation. 2002

6. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects
   J. S. Hulot and others. Blood. 2006

7. Patients with poor responsiveness to thienopyridine treatment or with diabetes have lower levels of circulating active metabolite...
   D. Erlinge and others. J Am Coll Cardiol. 2008

8. Cytochrome P450 polymorphisms and response to clopidogrel

9. Association of cyclooxygenase-1-dependent and -independent platelet function assays with adverse clinical outcomes in aspirin-treated patients...

10. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs
Aggregation of blood platelets by adenosine diphosphate and its reversal

G. V. R. Born

*Nature.* 1962;194:927-929

Required reading for anyone interested in platelet aggregation, this landmark article was the first to describe a method for evaluating adenosine diphosphate (ADP)-induced platelet aggregation that is still considered the gold standard today, nearly 50 years later. Because at the time it had been recently demonstrated that ADP could induce platelet aggregation in plasma, Born devised a technique to quantitatively follow the rate and extent of platelet aggregation.

Professor Born used human blood mixed with sodium citrate or heparin to prevent clotting. Blood was centrifuged for 20 minutes at room temperature to obtain platelet-rich plasma. The platelet-rich plasma was then transferred into transparent tubes to measure its optical density with an absorptiometer. Light at a wavelength of 600 μm was passed through the tubes. The scale of optical density was defined such that dark current was set at infinity and distilled water at zero. This method is now called light transmission, or optical, aggregometry, and light transmission aggregometers specifically designed for this purpose are usually used. The addition of ADP to platelet-rich plasma immediately caused the optical density to rapidly decrease as platelets aggregated. Moreover, the rate and extent of change in optical density was proportional to the dose of ADP added. However, when the dose of ADP added to platelet-rich plasma was too low, the initial drop in optical density observed was not maintained and reverted back to almost what it was at baseline after 10 minutes, suggesting that aggregation induced by ADP was potentially reversible.

Professor Born also observed that the aggregation induced by ADP could be reversed by adenosine monophosphate (AMP) or, to a lesser extent, by adenosine triphosphate (ATP). In the absence of AMP, the addition of ADP to platelet-rich plasma resulted in aggregation that was maintained for at least 20 minutes. When AMP was added to platelet-rich plasma 2 minutes before ADP, aggregation was prevented as the initial rapid decrease in optical density started to increase again within 2 minutes and was almost back to baseline level after 20 minutes.

As a result, it was proposed that AMP antagonized platelet aggregation induced by low doses of ADP and that the reversal of aggregation was “due to the disappearance of ADP presumably because it [was being] broken down to AMP, which, unlike ADP, does not cause platelets to aggregate.” It was only 3 years later that the second phase of aggregation was discovered.

It was also postulated, in a visionary way, that the inhibition of ADP-induced platelet aggregation by AMP may be the result of competition between ADP and AMP or ATP “for specific ‘aggregating sites’ on the platelets or for calcium or magnesium” and, as such, “some other substance could be used to inhibit or to reverse platelet aggregation in thrombosis.” Indeed, as was shown later, ADP binds to specific platelet receptors to induce aggregation, and agents that bind to these receptors are of the utmost importance in our current armamentarium of drugs to prevent coronary thrombosis.

The Beatles audition for EMI Records; The last soldiers of the French Foreign Legion leave Algeria; and Rwanda and Burundi become independent states.
Establishing the lowest possible dose of aspirin that can be administered chronically to inhibit platelet cyclooxygenase activity and thromboxane (TX) A₂ formation without interfering with the synthesis of renal prostaglandins (PGs) in healthy subjects was the purpose of the studies in this key article. To achieve this, the authors conducted two series of studies: a single-dose study and multiple-dose studies.

In the single-dose study, healthy volunteers were randomly administered aspirin at a dose ranging between 6 and 100 mg. Platelet TXA₂ production was measured after whole blood clotting in the form of serum TXB₂, a stable breakdown product of TXA₂. In doing so, the authors demonstrated that a single dose of aspirin caused a dose-dependent decrease in TXB₂ production, attaining 95% inhibition of TXA₂ formation with a 100 mg dose of aspirin.

In their multiple-dose studies, the authors tested a dose of 0.45 mg/kg daily, which corresponds, for this population, to an average dose of 33.3 mg. Like the 100 mg aspirin dose of the single-dose study, this low dose taken daily for seven days also caused 95% inhibition of serum TXB₂ production... but after four days of therapy!

Moreover, the long-term effects of low-dose aspirin on TXB₂ production were confirmed after a group of healthy volunteers took the 0.45 mg/kg dose of aspirin daily for one month. Similar to what was observed in the one-week study, "greater than 95% inhibition of platelet TXB₂ production was maintained throughout the whole treatment period." Upon discontinuation of aspirin therapy, platelet TXA₂ production returned to baseline levels over a similar time frame as "that described following a single 100 mg dose."

Because of a concern about the renal impact of cyclooxygenase inhibition with aspirin, the authors also assessed urinary excretion of PGE₂, PGF₂ₐ, and 6-keto-PGF₁₂ₐ, which reflect the renal synthesis of PGE₂, PGF₂ₐ, and prostacyclin (PGI₂). They showed that none were affected to any significant degree by 7 or 31 days of aspirin therapy. On the other hand, aspirin doses of 500 mg and 1 g daily for seven days did reduce the urinary excretion of the three renal PGs by 40%-60%. Finally, the lack of effect of low-dose aspirin on renal PG synthesis was further substantiated by the authors' furosemide study. Known to stimulate renin release through increased synthesis of renal PGI₂, furosemide was intravenously infused before and on two occasions during one-month low-dose aspirin therapy. As expected, the excretion rate of 6-keto-PGF₁₂ₐ and plasma renin activity followed the same time course under control conditions and during chronic aspirin treatment.

Of note, the “battle of the sexes” already existed back in 1982. The authors took care to compare the efficacy of low-dose aspirin in inhibiting TXA₂ formation between men and women, as previous investigators had suggested that the antithrombotic effect of aspirin was restricted to men. As expected, they “found no obvious difference in the extent, duration and selectivity of aspirin effects between healthy men and women.”

In view of this evidence, some people might be tempted to administer, in specific patients, even lower doses of aspirin than the standard 80-mg dose. One must keep in mind that the aspirin used in this study was plain acetylsalicylic acid powder incorporated into capsules. The amount of active ingredient reaching circulating blood after ingestion of these capsules may differ from that following administration of standard enteric-coated tablets.

1982

US President Ronald Reagan makes his first address to the UN General Assembly describing the Soviet Union as the “evil empire”; Prince William is born at St Mary’s Hospital in Paddington, London; and Argentine dictator Leopoldo Galtieri resigns following the Falklands War.
something already documented back in 1983 was the presence of cyclooxygenase, which contributes to the formation of different prostaglandins (PGs) with different actions, in various tissues. Prostacyclin (PGI\textsubscript{2}) had been recognized as a potent vasodilator and inhibitor of platelet aggregation. However, questions were arising regarding the sensitivity of various tissues to cyclooxygenase inhibition by aspirin. In this important paper, the authors examined the effects of aspirin on both thromboxane and PGI\textsubscript{2} synthesis in healthy individuals.

This was done in three steps. First, the authors measured urinary 2,3-dinor-6-keto-PGF\textsubscript{1α}, a metabolite of PGI\textsubscript{2}, in two male volunteers before, during, and after ingestion of aspirin 650 mg four times a day for one week. Excretion of 2,3-dinor-6-keto-PGF\textsubscript{1α} was reduced in both cases during aspirin administration and recovery of PGI\textsubscript{2} biosynthesis was delayed.

In the second phase of the study, FitzGerald and colleagues administered two regimens of aspirin in consecutive weeks to five healthy volunteers, starting with 20 mg daily for seven days, followed by 650 mg four times daily, also for seven days. This time, serum thromboxane (TX) B\textsubscript{2}, platelet function studies using Born’s method (see Summary 1), and bleeding time were measured in addition to 2,3-dinor-TXB\textsubscript{2} excretion, prior to dosing and on the final day of each treatment period. Whereas 2,3-dinor-TXB\textsubscript{2} excretion, serum TXB\textsubscript{2} formation, platelet aggregation, and serotonin release were decreased with the lower dose of aspirin, all were further inhibited by the higher dose.

Finally, in the last stage of the study, five healthy volunteers received consecutive one-week regimens of eight different doses of aspirin, ranging between 20 and 2600 mg daily. Urinary TX and PGI\textsubscript{2} metabolite concentrations were measured prior to the first dose of aspirin, on the final day of each dosing regimen, and for the three days following the last dose. Doses between 20 and 325 mg of aspirin led to a dose-dependent decrease in both TX and PGI\textsubscript{2} metabolites, whereas a plateau was observed at doses between 650 and 2600 mg. Interestingly, the fall in the concentration of PGI\textsubscript{2} metabolite reached statistical levels at a dose of 160 mg of aspirin and above, whereas a statistically significant decrease in TX metabolite was observed with only 80 mg of aspirin. On the other hand, the rate of recovery three days after cessation of aspirin was faster for TX than for PGI\textsubscript{2}.

The authors drew a number of conclusions from their results, including that “inhibition of thromboxane generation was greater than that of prostacyclin at all doses of aspirin tested.” In addition, “urinary thromboxane metabolite excretion was maximally inhibited at doses of 325 mg/day and above,” contributing to the standard practice of administering aspirin 325 mg daily to inhibit platelet aggregation and prevent acute coronary syndromes. Lastly, the demonstration by FitzGerald and colleagues of the distinctive effects of 80 and 160 mg of aspirin on TX and PGI\textsubscript{2} biosynthesis has certainly played a role in the presently popular use of low-dose aspirin.
Development of aspirin resistance in persons with previous ischemic stroke


Stroke. 1994;25:2331-2336

In previous years, although consideration had been given to the balance between thromboxane inhibition and prostacyclin biosynthesis, the authors were concerned that less attention had been paid to the dosage optimization of aspirin (ASA [acetylsalicylic acid]) for the prevention of recurrent ischemic stroke. In this key article, the authors reported on a cohort of patients with previous ischemic stroke whose biological markers of ASA antiplatelet efficacy over time they measured.

Over a period of 33 months, Helgason and colleagues recruited 306 patients taking ASA for prevention of recurrent stroke. These patients first underwent platelet aggregation studies according to the Born method (see Summary 1) using different agonists, two weeks after initiating ASA therapy. When inhibition of platelet aggregation was incomplete, the dosage of ASA was increased by 325 mg/day. This sequence was repeated every two weeks until complete inhibition of platelet aggregation was obtained or the maximal dose of 1300 mg/day was reached. When complete inhibition was attained, the patient was continued on that dosage and platelet aggregation was measured at 6-month intervals.

Upon initial testing, after two weeks of 325 mg daily (in most cases) of ASA, 34% of patients presented partial inhibition. At first glance, this number seems very high compared to what is usually reported in the current literature with regard to ASA resistance. However, the authors chose a somewhat “involved” definition in that in order to be considered to have full inhibition, patients had to show no platelet aggregation response to arachidonic acid and saline and limited response to adenosine diphosphate, epinephrine, and collagen.

Surprisingly, of those who initially demonstrated complete inhibition, 33% presented partial inhibition upon repeat testing. However, with dosage escalation, only 5% remained partially inhibited. On the other hand, of those who were initially only partially inhibited, 45% presented complete inhibition upon repeat testing 6 months later. Of these, 23% reverted back to partial inhibition over time when tested again. In the end, only 15% of those patients who were initially described as partial inhibitors maintained that status.

Notwithstanding these statistics, the most disturbing results of this article are the fluctuations that occur with a constant dosage of ASA in this patient population. Indeed, “of the 154 patients who were at one time on a dosage of ASA sufficient to completely inhibit platelet aggregation, 47 (30.5%) did not maintain that effect at repeated testing despite fulfilling the criteria of regular compliance checks.” Moreover, eight patients had a recurrence of stroke, and all of them had presented partial inhibition of platelet aggregation either upon admission for stroke or on previous testing.

The authors concluded by saying that “the possibility that ASA dosage needs to be adjusted to ensure continued efficacy, as is done with other medications such as warfarin, is clear.” Furthermore, “once antiplatelet therapy has been chosen for use to prevent recurrent ischemic stroke […] it may be desirable to ensure a measurable desired biological effect at the onset and at repeated intervals during the course of therapy.” Certainly, this approach has recently been promoted by various companies that now offer easy-to-use assays to measure inhibition of platelet aggregation induced by ASA. Whether dosage adjustments on the basis of the results obtained with these assays will improve clinical outcomes remains to be proven.

Ernesto Zedillo is elected President of Mexico; Russian troops are ordered into Chechnya by president Boris Yeltsin; and Japanese Crown Prince Naruhito announces his engagement to Masako Owada.
Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events

J. W. Eikelboom, J. Hirsh, J. I. Weitz, M. Johnston, Q. Yi, S. Yusuf

_Circulation_. 2002;105:1650-1655

Studies that deserve landmark status are few, but this article by Prof Eikelboom and colleagues is certainly one. For the first time, the authors demonstrated an association between “aspirin resistance” and the risk of cardiovascular outcomes.

The authors piggybacked a nested case-control study onto the large, randomized, controlled, 2 × 2 factorial design HOPE (Heart Outcomes Prevention Evaluation) trial. Their objective was to demonstrate an association between incomplete suppression of thromboxane (TX) generation, determined by urinary 11-dehydro-TXB2 levels, and increased risk of recurrent cardiovascular events in a high-risk population of patients, defined as ≥55 years with a history of myocardial infarction, stroke, peripheral vascular disease, or diabetes plus at least one other cardiovascular risk factor.

Of the 9541 patients included in the HOPE trial, data from 488 cases and the same number of controls were analyzed; the authors only included patients who were taking aspirin before randomization and at each follow-up visit and who provided an adequate baseline sample of urine. Cases were patients with confirmed myocardial infarction, stroke, or cardiovascular death after randomization; controls did not have any major cardiovascular events after randomization and were matched according to sex and age with cases.

Unsurprisingly, the authors did find that “the adjusted odds for the composite outcome of myocardial infarction, stroke, or cardiovascular death increased with each increasing quartile of baseline urinary 11-dehydro-TXB2 concentration […], with patients in the highest quartile having a risk 1.8-fold higher than those in the lowest quartile.” In fact, urinary concentrations of 11-dehydro-TXB2 upon inclusion into the trial were significantly higher in cases than in control patients, especially in those who had had a myocardial infarction or died of a cardiovascular cause. Obviously, this association was independent of conventional vascular risk factors. On the other hand, “baseline urinary concentrations of 11-dehydro-TXB2 were not significantly different between cases who had subsequent development of stroke and their matched control group.” The reason for the lack of association with the development of stroke was unclear for the authors, but it probably reflected a play of chance, considering the fact that aspirin did reduce the risk of stroke in high-risk patients and that elevated urinary concentrations of 11-dehydro-TXB2 had been reported in stroke patients previously.

In their discussion, the authors proposed several mechanisms to account for the incomplete suppression of TX synthesis by aspirin, some of which are still being discussed today. Polymorphisms or mutations of the cyclooxygenase 1 gene or upregulation of expression of cyclooxygenase 2, which can convert prostaglandin H2 to TXA2, can certainly contribute to observed “aspirin resistance.”

The authors’ conclusions draw attention to the “possibility that high urinary levels of 11-dehydro-TXB2 can prospectively identify patients who are relatively resistant to conventional antithrombotic doses of aspirin.” As expected, the AspirinWorks® test was later made commercially available. However, whether adjusting the dosage of aspirin in response to measured urinary 11-dehydro-TXB2 concentrations decreases the risk of cardiovascular events has yet to be proven.

The Netherlands legalizes euthanasia; Robert Steinhäuser shoots and kills 17 people at his school in Erfurt, Germany; and in excess of half a million people march on the streets of Caracas to protest against the Venezuelan government.
To produce the active metabolite responsible for blocking platelet P2Y12 receptors and to inhibit platelet aggregation, the prodrug clopidogrel needs to be activated via multiple biotransformation steps involving cytochrome P450 (CYP) isoenzymes, as detailed by the authors of this great proof-of-concept pharmacogenetic study. Unfortunately, the pharmacodynamic response of clopidogrel varies widely between patients and the causes of this variability were not totally clear in 2006, nor are they still today.

In addition to obvious causes like nonadherence, the authors postulated that genetic factors might significantly contribute to this phenomenon. Thus, they opted to perform a pharmacogenetic study looking at four functional polymorphisms found in genes encoding CYP isoenzymes that are involved in the metabolic transformation of clopidogrel, including CYP 3A4/5, 2C19, 2B6, and 1A2.

Twenty-five healthy young white men aged 18 to 35 years old were “literally” administered clopidogrel 75 mg daily for 7 consecutive days; the subjects had to present themselves at the laboratory every morning at 9 AM to receive and ingest clopidogrel in the presence of medical staff. They all underwent genotyping and platelet aggregation studies before, during, and after clopidogrel administration; platelet studies included light transmission aggregometry after stimulation with 10 µM adenosine diphosphate (ADP), and vasodilator-stimulated phosphoprotein (VASP) phosphorylation, a good indicator of P2Y12 activity.

The authors found that the single nucleotide polymorphisms selected for CYP2B6, CYP3A5, and CYP1A2 had no influence on clopidogrel response. “In contrast, the response to clopidogrel was strongly influenced by the CYP2C19 genotypic status.” Whereas platelet aggregation in response to 10 µM ADP was similar in the CYP2C19 wild-type homozygotes (*1/*1) and heterozygotes (*1/*2) at baseline, it was significantly lower after seven days of clopidogrel in the *1/*1, but not the *1/*2 subjects. This association was confirmed by VASP phosphorylation studies. The authors further stratified their subjects into quartiles based on the inhibition of platelet aggregation induced after seven days of clopidogrel treatment, to identify “poor responders.” Interestingly, “5 of the 7 subjects belonging to the first quartile of poor responders carried the CYP2C19*2 allele.”

In view of the results, the authors chose to study a posteriori four other functional CYP2C19 loss-of-function variants (*3, *4, *5, *6). Only one patient presented the CYP2C19*4 allele, in the heterozygous state, and he belonged to the third quartile. Notwithstanding, two *1/*2 carriers belonged to the second quartile and one to the third quartile. These results further clarify the impact of CYP2C19*2 polymorphism on clopidogrel response: others had demonstrated that inhibition of ADP-induced platelet aggregation was reduced in the presence of CYP2C19*2 polymorphism after 4 and 24 hours of a single 300 mg dose of clopidogrel. Now, we know that “the difference in ADP-induced platelet aggregation between genotypes persists after 7-day administration […] of clopidogrel” and “cannot be reversed by repeated dosing.”

As noted by the authors, the allelic frequency of CYP2C19*2 in whites is 15% and thus nonnegligible. However, “the results suggest that CYP2C19 *1/*2 subjects are not totally refractory to clopidogrel, but that they respond less well than CYP2C19 *1/*1 subjects.” Notwithstanding other genetic or nongenetic factors are also likely to influence clopidogrel responsiveness.

Smoke from forest fires in western Indonesia cause widespread pollution throughout the country and neighboring Malaysia; Russian journalist Anna Politkovskaya is found murdered in Moscow; and Roger Kornberg wins the 2006 Nobel Prize in Chemistry for his work exploring the molecular basis of eukaryotic transcription.
Patients with poor responsiveness to thienopyridine treatment or with diabetes have lower levels of circulating active metabolite, but their platelets respond normally to active metabolite added ex vivo

D. Erlinge, C. Varenhorst, O. O. Braun, S. James, K. J. Winters, J. A. Jakubowski, J. T. Brandt, A. Sugidachi, A. Siegbahn, L. Wallentin


As mentioned by Hulot and colleagues (see Summary 6), the causes of the high variability in the pharmacodynamic response to clopidogrel remain unclear. In their study, Erlinge and colleagues further addressed this issue. Pharmacodynamic poor responsiveness to clopidogrel was associated with worse clinical outcomes, regardless of which of the many definitions of insufficient platelet inhibition was used. Furthermore, diabetic patients had consistently been shown to be poor responders. The objectives of the authors were threefold: (i) to compare the incidence of pharmacodynamic poor responsiveness with clopidogrel and prasugrel, (ii) to characterize the pharmacokinetic profile of the active metabolites of clopidogrel and prasugrel in poor responders, and (iii) to evaluate P2Y₁₂ receptor function in poor responders.

One hundred and six patients completed this well-designed study. They were randomized in a double-blind, double-dummy, parallel group fashion to receive a loading dose of either clopidogrel 600 mg or prasugrel 60 mg followed by either clopidogrel 75 mg or prasugrel 10 mg once daily for 28 days. Light transmission aggregometry was performed after induction of platelet aggregation with adenosine diphosphate 5µM on multiple occasions, including baseline and day 29. Concentrations of active metabolites were measured, as was vasodilator-stimulated phosphoprotein (VASP) phosphorylation to evaluate P2Y₁₂ activity. Surprisingly, the incidence of pharmacodynamic poor responsiveness varied quite significantly depending on the definition used after both the loading dose and the maintenance dose, especially in clopidogrel-treated patients (between 9.6% and 92.5% after the loading dose, compared with between 0% and 16.4% in prasugrel-treated patients). On the other hand, and not surprisingly this time, the concentration of active metabolites was much lower in poorly responsive clopidogrel-treated patients, regardless of the definition of poor responsiveness used or the timing of evaluation (after loading or maintenance dose).

In an attempt to better decipher the origin of the reduced responsiveness to clopidogrel in some patients, the authors also evaluated the inhibition of P2Y₁₂ receptors by VASP phosphorylation at baseline and after addition of the active metabolite ex vivo in normal and poor clopidogrel responders. Interestingly, no difference was noted in the platelet reactivity index at baseline, nor after addition of the active metabolite either before the loading dose or during the maintenance dose, “indicating that the P2Y₁₂ receptor function and affinity for the [active metabolite] is similar for poor responders and good responders.”

Finally, the authors took a closer look at diabetic patients, who were significantly overrepresented among thienopyridine poor responders, regardless of the definition used, at most time points. They presented lower concentrations of active metabolites and higher platelet reactivity index, both after the loading and the maintenance doses compared with nondiabetic patients.

In view of these results, the authors conclude that “the impaired platelet inhibition in the clopidogrel poor-responder groups and in diabetic patients reflects lower plasma levels of active metabolite and not differences in platelet P2Y₁₂ receptor function.” As such, the absorption of the prodrug, in this case clopidogrel, its biotransformation into an active metabolite (see Summary 6), or both may contribute to lower levels of circulating active metabolites. “The reason that diabetic patients have lower levels of [active metabolites remains] unclear.”

The United Nations Food and Agriculture Organization estimates that 963 million people suffer from prolonged food deficiency globally; scientists report evidence of water vapor and carbon dioxide in the atmosphere of the extrasolar planet HD 189733; and the ruins of an ancient city belonging to the Wari culture are discovered in northern Peru.
Cytochrome P450 polymorphisms and response to clopidogrel


Now whereas the previous two studies delved into the possible causes of reduced responsiveness to clopidogrel in certain patients, Mega and colleagues conducted a superb study to investigate the influence of cytochrome P450 (CYP) polymorphisms on the pharmacokinetic and pharmacodynamic response of clopidogrel, and its effect on clinical outcomes. This study was done in two steps. Initially, the authors included 162 healthy subjects who participated in six studies and who received a loading dose of clopidogrel 300 mg. Plasma concentrations of the active metabolite were measured, as was the absolute reduction in maximal platelet aggregation from baseline, by light transmission aggregometry in response to adenosine diphosphate 20 µM; this was assessed 4 hours after clopidogrel administration. All patients were genotyped for 54 alleles from six CYP isoenzymes (CYP2C19, CYP2C9, CYP2B6, CYP3A5, CYP3A4, CYP1A2).

From this first cohort, the authors noted that carriers of at least one *CYP2C19* reduced-function allele (which corresponded to a third of the study population) demonstrated a 32% reduction in plasma exposure to the active metabolite and an absolute 9 percentage point reduction in maximal platelet aggregation in response to clopidogrel compared to noncarriers. Both responses were graded with further genotypic classification into ultrarapid, extensive, intermediate, and poor metabolizers. Pharmacokinetic and pharmacodynamic responses were of lesser magnitude in carriers of a reduced-function *CYP2B6* allele, whereas nonconsistent attenuation was observed with the other CYP genes.

In the second phase, the authors included 1477 patients with acute coronary syndromes undertaking planned percutaneous coronary intervention who provided a DNA sample. They were randomly assigned to the clopidogrel arm of the TRITON-TIMI 38 (TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis In Myocardial Infarction 38) trial, and received a 300 mg loading dose followed by a 75 mg daily maintenance dose for up to 15 months. In accordance with the results of the initial study, the “395 subjects carrying at least one *CYP2C19* reduced-function allele […] were at significantly higher risk for the primary efficacy outcome of death from cardiovascular causes, myocardial infarction, or stroke than were noncarriers” (hazard ratio, 1.53; *P*=0.01); the risk of stent thrombosis was also three times that among noncarriers.

In view of these results, Mega and colleagues concluded that their results strongly supported a link between genetic variations in *CYP2C19* and decreased concentrations of the active metabolite, reduced platelet inhibition, and lower protection against recurrent cardiovascular events in patients treated with clopidogrel. “Common polymorphisms in the *CYP2C19* gene, seen in approximately 30% of whites, 40% of blacks, and more than 55% of East Asians, significantly diminish both the pharmacokinetic and pharmacodynamic responses to clopidogrel and are “associated with adverse clinical outcomes, including a rate of death from cardiovascular causes, myocardial infarction, or stroke that is more than 50% greater and a rate of stent thrombosis that is greater by a factor of three than the rate of noncarriers”, these findings are troublesome! Indeed, the exponentially increased number of articles that have been published on this topic and on potential interactions between clopidogrel and *CYP2C19*-metabolized drugs over the past few months is a good reflection of the concern felt by many clinicians in this regard.

In response to the global economic crisis, German Chancellor Angela Merkel announces a €50 billion economic stimulus package; Barack Obama is inaugurated as the 44th President of the United States; and Iceland’s Minister of Business Affairs, Björgvin G. Sigurðsson, resigns as a result of the country’s financial crisis.
Association of cyclooxygenase-1-dependent and -independent platelet function assays with adverse clinical outcomes in aspirin-treated patients presenting for cardiac catheterization

A. L. Frelinger, Y. Li, M. D. Linden, M. R. Barnard, M. L. Fox, D. J. Christie, M. I. Furman, A. D. Michelson

Circulation. 2009;120:2586-2596

Cyclooxygenase 1 (COX-1) in platelets, when irreversibly acetylated, can diminish the risk of thrombotic events; this is how aspirin works and cuts the risk of these events by approximately 25% in high-risk patients. However, 10% to 20% of patients present a recurrent thrombotic event, despite being treated with aspirin. This may be the result of inadequate inhibition of platelet COX-1. A large number of different assays are available to estimate the incidence of aspirin resistance. Whether the results obtained from these tests can predict adverse clinical outcomes is what Frelinger and colleagues set out to find in this article.

Accordingly, the authors recruited 700 aspirin-treated patients presenting for diagnostic cardiac catheterization, as well as 36 aspirin-free healthy control subjects. All patients were administered 3 types of platelet function assays: (i) serum thromboxane (TX) B₂, a direct measure of platelet COX-1 function; (ii) 5 indirect measures of platelet COX-1 function, including platelet surface-activated glycoprotein IIb/IIIa, platelet surface P-selectin, monocyte-platelet aggregates, neutrophil-platelet aggregates—all after activation with arachidonic acid—and PFA-100 collagen-epinephrine (CEPI) closure time (CT); and (iii) PFA-100 collagen-adenosine diphosphate (CADP) CT, a COX-1 independent assay.

Patients were followed for 24 months and major adverse cardiovascular events (MACEs), including cardiovascular death, myocardial infarction, hospitalization for revascularization, and acute coronary syndrome, were observed in 20% of them. As expected, the authors found that a number of clinical variables were independently associated with an increased risk of subsequent MACE.

In terms of platelet function, the authors noted reductions in serum TXB₂ and in all four arachidonic acid–stimulated indirect measures of COX-1 function, and a prolongation in PFA-100 CEPI CT in aspirin-treated patients compared with aspirin-free healthy controls. However, the PFA-100 CADP CT was not different to that of aspirin-free subjects, confirming “that the PFA-CADP is COX-1 independent.”

Interestingly, only one of the COX-1-dependent assays, serum TXB₂ >3.1 ng/mL, showed a significant association with MACE; none of the indirect COX-1-dependent tests were associated with MACE. But surprisingly, MACE occurred more frequently in patients presenting PFA-100 CADP CT <65 seconds. This suggests that “poor outcomes in aspirin-treated patients are better correlated with platelet reactivity to collagen, ADP and shear (all of which are measured by the PFA-100 CADP CT) rather than with residual COX-1 function.” In addition, as expressed by the authors, these results raise the possibility that PFA-100 CADP may be a useful tool to predict outcomes in patients with coronary artery disease and that “greater inhibition of ADP-induced platelet activation would result in improved outcomes in this patient group,” which is supported by results of TRITON-TIMI 38 (TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet inhibition with prasugrel-Thrombolysis In Myocardial Infarction 38).

On the basis of these results, Frelinger and colleagues conclude that “in this patient population, poor clinical outcomes […] are due in part to incomplete COX-1 inhibition, but are also due in part to COX-1-independent platelet reactivity.” Accordingly, increasing the dosage of aspirin would probably not be very effective in decreasing the risk of MACE because multiple factors, in addition to COX-1 inhibition, seem to play a significant role in the development of these events. Therefore, the term “aspirin resistance” is a misnomer, and its use should be avoided.

The 25th anniversary of the Bhopal disaster is marked in India; the Nepalese cabinet meets on Mount Everest to highlight the impact of climate change on the region; and evidence of mass cannibalism during the Neolithic period is uncovered at a 7000-year-old burial site in Germany.
Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs

J. R. Vane

*Nat New Biol.* 1971;231:232-235

Even though aspirin had been used for close to a century, its mechanism of action remained hidden until 1971 when Vane identified that “some of the therapeutic effects of sodium salicylate and aspirin-like drugs are due to inhibition of the synthesis of prostaglandins.”

Vane, with the help of Piper, had previously found a substance they called “rabbit aorta contracting substance” or RCS. RCS had a short half-life (<5 minutes), but its chemical nature was unknown. Arachidonic acid was known as a prostaglandin (PG) precursor able to induce bronchoconstriction and to release RCS from perfused lungs. Vane hypothesized that RCS was a PG or an intermediate structure between arachidonic acid and PGE₂ or PGF₂α, and that anti-inflammatory substances such as aspirin could inhibit the enzyme responsible for PG synthesis.

To prove his theory, Vane excised lungs from four adult guinea pigs. Lung tissue was homogenized, centrifuged and then incubated with arachidonic acid. Varying amounts of indomethacin, sodium acetylsalicylate, and sodium salicylate were added to the preparation of homogenate and arachidonic acid and shown to elicit inhibition of PGF₂α activity in a log-linear relationship. Indomethacin was 47-times more potent than aspirin on a molar basis, whereas sodium salicylate was less potent than aspirin. Similar results were obtained with respect to PGE₂.

In a second experiment, Vane confirmed that indomethacin and aspirin inhibited the synthesis of PGE₂ and PGF₂α. He used lower concentrations of the two drugs, which he added to lung homogenates incubated with arachidonic acid. Once again, PGE₂ and PGF₂α activity was reduced with both agents, although, as expected, in different proportions.

Although Vane had now proven that aspirin-like drugs inhibited PG synthesis, the mechanism of this inhibition was still unknown. As Vane cleverly theorized: “It is not known how the inhibition is brought about. If it is by competition with arachidonic acid for the active site of the enzyme, this might explain why all of these anti-inflammatory substances contain an acidic group. It would also explain why hydrocortisone, an anti-inflammatory substance of a different type, has little or no inhibitory action against the prostaglandin synthesizing enzyme(s).”

In his discussion, the author presented supporting evidence that the three principal actions of anti-inflammatory acids were explained by a direct inhibition of PG synthesis. First, PGE₁ induces fever that cannot be antagonized by the antipyretic drug 4-acetamidophenol (more commonly known nowadays as acetaminophen); inhibition of PGE₁ could explain the antipyretic action of aspirin-like drugs. Second, PGs such as PGE₁, PGE₂, PGF₁α, and PGF₂α have been isolated from fluid perfusing the skin of patients with allergic eczema; hence, inhibition of PG synthesis by this group of substances supports their anti-inflammatory action. Finally, PG infusion induces headache; the relief of headache by aspirin and indomethacin may be explained by an inhibition of PG synthesis.

In 1982, Vane, along with Sune K. Bergström and Bengt I. Samuelsson, was awarded the Nobel Prize in Physiology or Medicine “for their discoveries concerning prostaglandins and related biologically active substances.” Later research showed that aspirin-like drugs work by inhibiting cyclooxygenase, the enzyme responsible for the conversion of arachidonic acid into “rabbit aorta contracting substance,” now better known as thromboxane A₂.

Neville Bonner becomes the first indigenous Australian to sit in the Parliament of Australia; Harold Lloyd, the American actor and filmmaker, dies; and Ray Tomlinson sends the first ARPAnet e-mail between host computers.
Aspirin Resistance

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selected by Carlo Patrono, MD
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