Humans require rapidly responding, tightly regulated hemostasis because of their closed high-pressure circulatory system. Minor variation in response may predispose to pathological bleeding or thrombosis. In the appropriate setting, pharmacological intervention with antiplatelet therapy stabilizes the atherothrombotic phenotype, though with concomitant hemorrhagic risk. Populations with favorable risk–benefit ratios for acetylsalicylic acid (aspirin) and clopidogrel therapy have nevertheless been defined in major clinical trials. Treatment benefit is established for secondary prevention of cardiovascular and cerebrovascular events, management of acute coronary syndromes, and as an adjunct to percutaneous and surgical revascularization. There is evidence, however, that not all individuals respond comparably to antiplatelet drugs and hence the concept of aspirin and clopidogrel “resistance” has arisen. The term is misleading to antiplatelet drugs and hence the concept of aspirin and clopidogrel “resistance” has arisen. The term is misleading though because there are many determinants of failure to respond to treatment.

Clinical Imperative for Consistent Platelet Inhibition

Consistent levels of platelet inhibition are required to deliver effective therapy. Adverse consequences of variable response are particularly apparent when antiplatelet drugs are used as an adjunct to coronary revascularization. During percutaneous coronary intervention (PCI), atherosclerotic plaque is invariably disrupted, thrombosis occurs, and endothelial healing is delayed. Intensive periprocedural platelet inhibition minimizes morbidity and mortality, whereas persistence of a prothrombotic environment necessitates chronic antiplatelet therapy. Failure to provide adequate platelet inhibition in all individuals can result in stent thrombosis, myocardial infarction, and death. Platelet inhibition with aspirin at the time of coronary artery bypass graft surgery also provides benefit. Yet aggressive therapy with aspirin and clopidogrel combined may increase perioperative bleeding in some cases. These contrasting clinical problems underlie the need for a tailored approach to therapy and illustrate the requirement for consistent levels of platelet inhibition and a means to confirm individual response.

Platelet Adhesion, Activation, and Aggregation

Platelets adhere to sites of vascular injury; however, endothelial disruption is not a prerequisite. Atherosclerotic lesions are associated with impaired endothelial function and hence are susceptible to platelet and leukocyte adhesion. Indeed, patients with atherosclerosis have enhanced baseline platelet activation, which is reflected by corresponding increases in urinary thromboxane (TX) metabolite excretion. Initially, platelets tether to the vessel wall via membrane integrins and selectins. Subsequent rolling and firm adhesion has been demonstrated by intravital microscopy in experimental models of microvascular injury. Shear stress augments adhesion receptor engagement and platelet activation (so-called “outside-in” signaling). This in turn triggers release or generation of soluble platelet activators such as TX, adenosine diphosphate (ADP), and thrombin. A layer of activated platelets forms and attracts other platelets and leukocytes. This is followed by either stable thrombus formation or rapid resolution.

Activated platelets release inflammatory and mitogenic proteins that promote leukocyte chemotraction, vascular inflammation, and further modify the endothelial phenotype. Indeed, there is growing evidence that platelet adhesion is involved in the earliest development of atherosclerotic lesions. On activation, the most densely expressed platelet integrin αIIbβ3 (glycoprotein [GP] IIb/IIIa), undergoes conformational change, binds soluble fibrinogen and von Willebrand factor, and facilitates platelet aggregate formation. Notably, GP IIb/IIIa gradually loses its binding capacity when platelets are stimulated by ADP alone. However, more potent agonists such as thrombin induce persistent fibrinogen binding. The cycle of initiation, propagation, and perpetuation of platelet activation creates the platelet mass that forms a nidus for coagulation. Fibrin generation and release of secondary platelet agonists propagate this process. Secondary agonists continuously activate integrins and importantly may be required to prevent disassembly of the early platelet aggregate. Soluble ADP, TXA2, soluble CD40 ligand, and the product of growth arrest specific gene 6 are prominent in these paracrine signaling pathways.

Platelet Signaling and Thromboxane

Thromboxane and Its Platelet Receptor

Arachidonic acid is released from membrane phospholipids in response to most platelet agonists. Hydrolytic cleavage...
follows activation of the enzyme phospholipase A_2. On release, arachidonic acid is rapidly metabolized by prostaglandin (PG) H_2 synthase, also known as cyclooxygenase (COX). Platelet COX converts arachidonic acid via PGG_2 to PGH_2. In turn, PGH_2 is converted to an unstable, biologically active intermediate TXA_2, by the downstream enzyme TX synthase. TXA_2 activates the platelet via the cell membrane G-protein–coupled TX (TP) receptor. Notably, activation of the TP receptor causes irreversible platelet aggregation in part through ADP release and subsequent platelet activation. Collagen, thrombin, and ADP all induce TXA_2 synthesis and release by platelets. Inhibitors of COX prevent platelet aggregation in response to arachidonic acid. They also inhibit second-wave aggregation in response to weak platelet agonists such as epinephrine, low concentration collagen, and ADP, but not to potent agonists like thrombin.

**Acetylsalicylic Acid (Aspirin)**

**Aspirin and Cyclooxygenase-1**

There are at least 2 isoforms of the COX enzyme, COX-1 and COX-2. Both are membrane-bound homodimeric molecules, although mouse studies suggest that PGH_2 synthase–1 and PGH_2 synthase–2 may heterodimerise. COX-1 is constitutively expressed and regulates house-keeping cellular functions such as vascular hemostasis, gastric mucosal integrity, and renal blood flow. COX-2 is largely absent from normal tissues; however, it is induced by cytokines and growth factors to regulate inflammation and cell growth. COX-1 and COX-2 coexist in the vasculature and macrophages, and expression is induced in atherosclerotic plaque. Both isoforms are present in mature megakaryocytes, but mature platelets predominantly express COX-1. In conditions of high platelet turnover, a proportion of platelets may also express COX-2.

Aspirin covalently modifies both COX-1 and COX-2, although its affinity for COX-1 is 50 to 100 times that for COX-2. Aspirin acetylates a serine hydroxyl group at position 529 in a narrow region of COX-1’s hydrophobic pocket and thereby sterically inhibits the passage of arachidonic acid to the so-called active site of the enzyme. Platelets are anucleate cytoplasts and largely lack transcriptional activity. Therefore, aspirin induces an irreversible defect in TX synthesis, which persists for the lifespan of the platelet (8 to 10 days). Only 10% of the platelet pool is replenished daily, so despite the short half-life of aspirin (15 to 20 minutes), plain low-dose aspirin can fully inhibit platelet COX-1 on repeat daily dosing. Inhibition of TX biosynthesis is understood to be the principal mode by which aspirin prevents vascular thrombosis. This apparently dose-independent effect on platelet function contrasts with the clearly dose-dependent aspirin-induced gastrointestinal toxicity.

**Platelet Capacity for Thromboxane Synthesis**

Being anucleate, the platelet has finite capacity to generate TX; however, in vivo biosynthesis varies considerably. Capacity for platelet TX synthesis in response to physical and chemical stimuli is approximately 1000-fold greater than endogenous plasma levels. Interestingly, TX biosynthesis in patients with stable coronary artery disease (CAD) is similar to that of healthy individuals. However, patients with greater atherosclerotic burden, such as those with severe peripheral vascular disease, have markedly increased in vivo TX biosynthesis. Enhanced platelet activation and de novo TX biosynthesis by vascular cells and monocytes may contribute to what is largely a COX-1–mediated process. Phasic increases in TX synthesis occur in subjects with unstable angina and acute stroke, and occur during PCI, which presumably reflects transiently increased platelet activation.

**Inhibition of Thromboxane Generation and Platelet Aggregation by Aspirin**

Aspirin inhibits in vitro platelet aggregation triggered by exogenous arachidonic acid (metabolized to TXA_2) and low-dose ADP, but not platelet response to stronger agonists such as thrombin. Capacity of platelets to generate TXA_2 can be estimated by the measurement of its stable metabolite TXB_2 in blood clotted at 37°C for 45 minutes. Aspirin inhibits serum TXB_2 formation in a dose-dependent manner; however, 95% inhibition is the minimum required to achieve full platelet inhibition. Indeed, the relationship between serum TXB_2 level and suppression of platelet aggregation is nonlinear, and maximum inhibition of aggregation and prolongation of the bleeding time may require 99% serum TXB_2 inhibition (Figure). It is important, therefore, to note that minimal residual capacity to generate TX may be enough to sustain TX-dependent platelet activation. Thus, although low concentrations of the TX analog U46619 or epinephrine alone may fail to activate aspirin-treated platelets, inhibition is overcome when the 2 agonists are combined. Consistent with these findings, 99% inhibition of serum TXB_2 was required to suppress platelet aggregation fully in a population with stable CAD.

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Aspirin to Prevent Cardiovascular Disease

The role of aspirin in secondary prevention of cardiovascular disease is well established. A recent meta-analysis concluded that aspirin therapy reduces the combined end point of serious vascular events by one quarter, nonfatal myocardial infarction by one third, nonfatal stroke by one quarter, and vascular events by one quarter, nonfatal myocardial infarction, the number of vascular events avoided with aspirin therapy is approximately 100 times the number of major infarction, the number of vascular events avoided with aspirin.

In the context of myocardial infarction, the number of vascular events avoided with aspirin therapy is approximately 100 times the number of major hemorrhagic complications. Absence of benefit when aspirin is used for primary prevention of cardiovascular events presumably reflects the narrower risk-to-benefit ratio in this setting. A primary preventative role in higher risk subpopulations remains to be established.

Variable Platelet Response to Aspirin

Treatment Failure

Aspirin does not prevent the majority of cardiovascular events. This is not surprising because aspirin blocks only one of several pathways of platelet activation and aggregation. In some cases however, failure to respond to aspirin may be caused by an inadequate primary pharmacological effect. This has sometimes been referred to as "aspirin resistance." Depending on the population studied, the assay used, and the definition applied, prevalence of aspirin resistance is estimated to be between 5% and 65%. Disparity in the reported frequency of aspirin resistance reflects the diverse nature of the populations studied, the wide variety of tests used, and the arbitrary cut-off values imposed (Table 1).

Measures of Aspirin Response

Platelet Function Assays

Pharmacokinetic studies of aspirin are not particularly informative. Aspirin is unstable and rapidly hydrolyzed to salicylate, which is an inactive and more stable product. This conversion occurs initially in the gut, so plasma salicylate is a poor measure of drug bioavailability. Aspirin exerts much of its effect in the presystemic circulation before its inactivation in the liver. Thus, it may have had an antiplatelet effect despite failure to detect aspirin in the systemic circulation.

Platelet response to aspirin can be determined with the use of a variety of assays (Table 1). Most assays measure response to agonists in vitro. Weak agonists or low concentration of strong agonists depend on platelet TX generation to produce aggregation. Similarly, platelet aggregation to exogenous arachidonic acid is dependent on TX generation. Incomplete inhibition of arachidonic acid–induced platelet aggregation, or failure to prevent the TX-dependent second wave of platelet aggregation in response to weak agonists, indicates incomplete platelet COX inhibition.

Thromboxane Assays

The primary pharmacological effect of aspirin, which is understood to prevent thrombosis, is almost complete inactivation of platelet COX-1 and consequent inhibition of TX biosynthesis. Assays that detect platelet COX-1 function best represent aspirin response. Ex vivo determination of TXB2 in serum reflects maximal capacity of activated platelets to synthesize TX via the COX-1 pathway and is a sensitive measure of aspirin response. Levels of the urinary TX metabolite 11-dehydro-TXB2 reflect in vivo TX biosynthesis. Though less specific for TX generated by platelet COX-1, this assay has been correlated with clinical outcome. The relationship between serum TXB2 and 11-dehydro-TXB2 in urine is nonlinear, and profound continuous inhibition of the former is necessary to suppress the urinary metabolite. This nonlinear response may reflect the contribution of extraplatelet (vascular and renal) TX sources or TX generated by COX-2. Plasma levels of TXB2 are very low, and plasma assays generally lack the sensitivity and specificity to estimate the effect of aspirin. Of greater concern is the fact that plasma TXB2 levels are readily confounded by inadvertent ex vivo platelet activation, which occurs readily during sample collection and processing.

Platelet Aggregation Assays

Inhibition of platelet aggregation is frequently used to measure antiplatelet response. Multiple agonists of varying concentrations have been used to assess aspirin response. Different agonists, however, reflect COX-1–dependent platelet activation to varying degrees. Arachidonic acid is the substrate for COX-1–dependent TX generation in platelets, so aggregation response closely reflects platelet COX-1 activity. The inhibitory effect of aspirin on arachidonic acid–induced platelet aggregation, however, is nonlinear and may reflect release of secondary agonists that act in synergy with TX. This finding may also explain the modest correlation observed between serum TXB2 levels and arachidonic acid–induced platelet aggregation in patients with stable CAD (Figure). However, arachidonic acid–induced platelet activation ex vivo correlates with baseline circulating platelet activity, which suggests that it does parallel in vivo platelet activation.
IIb/IIIa antagonists, may prove clinically useful.

bleeding, be it sensitive to aspirin, thienopyridine, or GP

Ultimately, a sensitive and specific, yet rapid and inexpensive

required, so these devices currently remain research tools.

correlation with clinical outcome in large prospective trials is

assays to determine drug response (Tables 1 and 2). However,

bined platelet agonists, agglutination to fibrinogen-coated

beads, and adhesion and aggregation under arterial flow

conditions. Small studies have explored the utility of these

assays need to be performed using facilities that are not

widely available.

Flow Cytometry

Surface expression of P-selectin and activated GP IIb/IIIa

receptor by flow cytometry may also be used to determine

platelet inhibition by aspirin or clopidogrel. However, these

assays need to be performed using facilities that are not

widely available.

Semiautomated Point-of-Care Assays

Advent of newer antiplatelet drugs and emergence of the

concept of aspirin and clopidogrel resistance coincide with

the development of semiautomated point-of-care platelet

function assays. Potential advantages of these systems in-
Mechanisms That Underlie Incomplete Platelet Response to Aspirin

Incomplete platelet response to aspirin, so called aspirin resistance, likely reflects a composite of processes. These can broadly be divided into pharmacokinetic or pharmacodynamic mechanisms. Pharmacokinetic determinants of an incomplete aspirin response include noncompliance, inadequate dosing with various aspirin formulas, and interactions with other COX inhibitors. Pharmacodynamic factors result from failure to inhibit platelet COX despite adequate plasma levels. Enhanced platelet turnover, transcellular metabolism of PG precursors, and genetic variants of COX-1 may obviate platelet COX inhibition. Isoprostanes, which are non–enzymatic oxidation products of arachidonic acid, may activate the platelet TP receptor, which thereby directly circumvents COX inhibition.

Drug Compliance

When failure to respond to aspirin is assessed, noncompliance with therapy must be assumed from the outset. Regardless of disease process, prognosis, or symptoms, many patients routinely miss medication doses. A recent study of patients recovering from ischemic stroke showed that >10% were noncompliant with aspirin. Clinical implications of aspirin noncompliance have been studied in patients with prior myocardial infarction. Noncompliance, detected by serum TX assay and on interview, occurred in 16% of the population and was associated with 4-fold higher incidence of death, reinfarction, or rehospitalization at 12 months of follow-up. Others have associated aspirin withdrawal for any reason with hospitalization for an acute coronary syndrome and specifically late stent thrombosis.

Aspirin Dose

Recommended drug doses are generally based on population rather than individual dose-response analysis, and considerable interindividual variability occurs. Dose-dependent variability in platelet response to aspirin has been determined with various biochemical assays. Indeed, there is evidence to suggest that response to low-dose aspirin varies with anatomic distribution of atherothrombosis. Secondary prevention studies in large populations, however, fail to show

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Subjects (n; % Female; Mean Age)</th>
<th>Clopidogrel Dose (Mean Follow-Up Period)</th>
<th>Clopidogrel Resistance Assay (Prevalence)</th>
<th>Clinical Outcome Associated With Clopidogrel Resistance (Assay Results)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barragan et al (2003)</td>
<td>Stent thrombosis (&lt;30 days) vs no stent thrombosis (48 [16 cases]; 26; 67 y)</td>
<td>Clopidogrel 75 mg twice daily or ticlopidine 250 mg twice daily</td>
<td>Flow cytometric assay VASP phosphorylation (% platelet reactivity)</td>
<td>Platelet reactivity in patients with stent thrombosis vs no stent thrombosis (63.2% ± 9.56% vs 39.8 ± 10.9%; P&lt;0.0001)</td>
</tr>
<tr>
<td>Mobley et al (2004)</td>
<td>Patients on PCI (50, 20, 58 y)</td>
<td>75 mg daily maintenance; 300 mg loading clinician’s discretion (6 m)</td>
<td>Optical aggregometry 1 μmol/L ADP; &lt;10% average platelet inhibition (30%)</td>
<td>No correlation with major adverse clinical events</td>
</tr>
<tr>
<td>Matetzky et al (2004)</td>
<td>Patients on primary PCI for acute STEMI (60, 20, 58 y)</td>
<td>300 mg load post-PCI, then 75 mg daily × 3/12 (6 m)</td>
<td>Optical aggregometry 5 μmol/L ADP (first quartile comparison to remainder)</td>
<td>Recurrent cardiovascular events (40% vs 6.7%; P=0.007)</td>
</tr>
<tr>
<td>Gurbel et al (2005)</td>
<td>Patients on PCI (192; 44; 61 y)</td>
<td>300 mg or 600 mg loading, 75 mg daily (6 m)</td>
<td>Optical aggregometry 5 and 20 μmol/L ADP TEG hemostasis analyzer (upper quartile comparison to remainder)</td>
<td>Ischemic events (upper quartile) ADP aggregation (63 ± 12% vs 56 ± 15%, P=0.02) Clot strength (74 ± 5 mm vs 65 ± 4 mm, P&lt;0.001) Time to fibrin generation (4.3 ± 1.3 minutes vs 5.9 ± 1.5 minutes, P&lt;0.001)</td>
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<tr>
<td>Azenberg et al (2005)</td>
<td>Stent thrombosis vs no stent thrombosis (32 [10 cases]; 85; 58 y)</td>
<td>300 mg load post-PCI, then 75 mg daily × 3/12</td>
<td>Coaxial cylinder shearing device Shear-induced platelet aggregation</td>
<td>Shear 200 S⁻¹ (40.9 ± 12.2% vs 18.2 ± 18%, P=0.013) Shear 4000 S⁻¹ (57.4 ± 16.4% vs 23.4 ± 21.2%, P=0.009)</td>
</tr>
<tr>
<td>Gurbel et al (2005)</td>
<td>Stent thrombosis vs no stent thrombosis (120 [20 cases]; 43; 63 y)</td>
<td>75 mg daily ≤300 mg loading</td>
<td>Optical aggregometry 5 and 20 μmol/L ADP P2Y12 reactivity ratio by VASP phosphorylation Flow cytometry assay of GP IIb/IIIa expression (upper quartile comparison to remainder)</td>
<td>5 μmol/L ADP aggregation (49 ± 4% vs 33 ± 3%, P&lt;0.05) 20 μmol/L ADP aggregation (65 ± 3% vs 51 ± 2%, P&lt;0.001) P2Y12 reactivity ratio (69 ± 5% vs 46 ± 9%, P=0.03) Mean fluorescence intensity for stimulated GP IIb/IIIa expression (138 ± 19 vs 42 ± 4, P&lt;0.001)</td>
</tr>
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</table>

GP indicates glycoprotein; STEMI, ST-elevation myocardial infarction; and VASP, vasodilator-stimulated phosphoprotein.
additional clinical benefit of higher aspirin doses.23 Indeed, evidence that gastrointestinal injury increases as aspirin dose exceeds the dosage required for an antiplatelet effect, as well as the increasing prescription of combined antiplatelet therapy, may underlie the recent downward revision of recommended aspirin maintenance doses in patients with CAD.

Aspirin Formulation
Initial aspirin dose finding studies were performed with plain aspirin, which is rapidly absorbed from the stomach and small intestine, has a bioavailability of about 50% and achieves peak plasma levels in 30 to 40 minutes. It is then rapidly inactivated in the liver and gut and excreted mainly in urine. Platelet exposure and COX inhibition occur initially in the portal circulation, and as a result, antiplatelet activity has occurred before aspirin enters the systemic circulation. As a consequence of slow platelet turnover, doses of plain aspirin as low as 30 mg inhibit platelet TX formation in healthy subjects.9 Indeed, a sophisticated controlled release aspirin was developed to limit aspirin activity to the portal circulation and thus spare systemic PG12 biosynthesis.49

It is assumed that all low-dose aspirins are created equal; however, there is evidence to the contrary. “Aspirin” now encompasses a myriad of formulations; various salts, polymer-coated, controlled or rapid-release (compressed, soluble), buffered and enteric-coated preparations. Indeed, low-dose enteric-coated aspirin preparations are increasingly prescribed in an attempt to reduce gastrointestinal side effects. However, differences in formulation influence bioavailability of a drug that is now administered in critically low doses to individuals who respond variably. Plain preparations release aspirin (a weak acid, pKa=3) into the acidic environment of the stomach where it is protected from deacetylation, remains nonionized and lipid-soluble, and thus is rapidly absorbed. Enteric-coated preparations, however, deliver aspirin into the almost neutral pH environment of the small intestine where absorption is delayed (peak plasma levels occur in 2 to 4 hours), and bioavailability is reduced.17,35 Studies among healthy volunteers and patients with stable CAD indicate that some subjects treated with low-dose enteric-coated aspirin fail to achieve minimum thresholds of effective platelet inhibition (>95% serum TXB2 inhibition).11 An inverse relationship between patient weight and level of platelet inhibition was detected in both populations. Among healthy volunteers with a suboptimal treatment response, superior platelet inhibition was demonstrated with plain aspirin. In patients with stable CAD, younger heavier subjects and those with a history of prior myocardial infarction were most likely to have evidence of incomplete COX inhibition.17,35

Pharmacodynamic Interaction With Nonsteroidal Antiinflammatory Drugs
Some nonsteroidal antiinflammatory drugs may interact with aspirin and interfere with its antithrombotic effect. Inhibitors of COX-1 such as ibuprofen and naproxen share a common docking site with aspirin and prevent acetylation of aspirin’s target serine residue within the hydrophobic pocket of the enzyme.50 Indeed, use of high-dose nonselective nonsteroidal antiinflammatory drugs by patients who take aspirin for secondary prevention has been linked to adverse cardiovascular events.51 Although medical professionals are increasingly aware of this potential interaction, direct access to over-the-counter nonsteroidal antiinflammatory drugs is difficult to regulate.

Enhanced Platelet Turnover, COX Regeneration, and Aspirin-Insensitive Eicosanoid Biosynthesis
Regeneration of COX-1 and COX-2 occurs in conditions associated with enhanced platelet turnover and may overcome the inhibitory response to aspirin.8 Continued TX formation despite aspirin therapy was detected in patients after coronary artery bypass graft surgery.52 Addition of terbogrel, a combined TX synthase and TP receptor inhibitor, further reduced TX generation. Platelet COX-2 was also detected; however, selective inhibition of COX-2 did not prevent TX generation, which points to incomplete inhibition of the COX-1 pathway as the mechanism that underlies persistent TX formation.

Mature platelets are anucleate and therefore should not be able to regenerate COX. However, a recent study introduced the novel concept that platelets may splice endogenous pre-mRNA in response to external signals. Thus, platelets may have the ability to translate mature mRNAs into biologically active proteins and thereby regenerate COX-1 de novo in response to cellular activation.53 In a study of healthy volunteers, TXA2 biosynthesis in response to thrombin and fibrinogen recovered in a time-dependent manner and was abrogated by translational inhibitors such as rapamycin.54 This finding may explain observed temporal trends toward loss of platelet inhibition despite chronic aspirin therapy.55

Mechanisms have been proposed in which platelet TX is generated despite COX-1 inhibition. Precursors of PGH2 generated by vascular tissue and metabolized by platelet TX synthase may bypass platelet COX inhibition. Such transcellular metabolism could occur at sites of atherothrombosis or via platelet-leukocyte aggregates. More simply, local release of vascular TX or PG endoperoxides may activate the platelet TP receptor and act in synergy with weak platelet agonists such as epinephrine or subthreshold levels of stronger agonists.56 A recent study detected arachidonic acid–induced platelet activation independent of COX activity that was partially mediated by ADP.56 Finally, isoprostanes generated nonenzymatically by arachidonic acid oxidation are insensitive to aspirin, yet can partially activate the TP receptor in a COX-independent manner.56

Enhanced Platelet Aggregability and Genetic Determinants of Aspirin Response
Variation in genes, which encode enzymes or receptor targets of antiplatelet drugs, may modulate pharmacological response. In effect, genetic variation in any platelet signaling component, whether directly targeted by a drug or not, has the potential to influence antiplatelet response. COX-1 haplotype modulates platelet response to aspirin determined by in vitro platelet function assays.57 The precise mechanism involved, be it modulation of COX-1 enzyme expression, biochemical function, interaction with pharmacological agents, or an unrelated process, remains to be established.
Thienopyridines (Ticlopidine and Clopidogrel)
Adenosine Diphosphate and Its Platelet Receptor
ADP is released actively from platelet-dense granules and passively by damaged erythrocytes and vascular cells. It activates platelets via 2 surface-expressed G-protein–coupled receptors, P2Y1 and P2Y12. Each acts through a distinct signaling cascade, and coordinated activation of both is required to induce full platelet aggregation. At low concentrations, ADP is a relatively weak agonist whose activity is reinforced by platelet synthesis of TXA2, which causes granule secretion and secondary platelet aggregation.68 Soluble ADP, however, amplifies response to other platelet agonists, which makes it an important drug target. Signaling via P2Y1 induces platelet shape change, reversible aggregation, and initial GP IIb/IIIa activation. Signaling through P2Y12 perpetuates GP IIb/IIIa activation, maintains its high affinity state, and appears critical for stable platelet aggregate formation. Importantly, P2Y12 antagonism may not only prevent platelet aggregation but also promote disaggregation.69 Indeed, hereditary human ADP receptor deficiency results in a mild hemorrhagic phenotype characterized by prolongation of the bleeding time, impaired platelet aggregation, and spreading and formation of unstable platelet aggregates.59 Mouse models of P2Y1 or P2Y12 deficiency demonstrate impaired platelet aggregation to ADP, TX/endothelium-derived hyperpolarizing factor analogs, and thrombin, particularly at low agonist concentrations.60

The role of the ADP receptor extends beyond platelet activation. Antagonism of P2Y12 may also attenuate CD40L and P-selectin expression, inhibit platelet-leukocyte aggregate formation, and abrogate periprocedural rise in C-reactive protein in patients who undergo revascularization. Furthermore, both ADP receptors have also been linked to rapid activation of intravascular tissue factor, the main initiator of physiological coagulation and a central component of pathological thrombosis.61 Thus, ADP antagonism may modulate coagulation and vascular inflammation in addition to platelet thrombosis.

Ticlopidine and Clopidogrel
Ticlopidine and clopidogrel block the ADP pathway and suppress its amplifying effect on platelet response to other agonists. Both agents inhibit platelet aggregation induced by ADP, TX analogs, collagen, low-dose thrombin, and shear, but strong agonists such as high-dose thrombin can overwhelm ADP inhibition. Both drugs prolong the bleeding time (1.5- to 2-fold longer than baseline), impair clot retraction, and render thrombin-induced platelet aggregates susceptible to disaggregation.

Ticlopidine and clopidogrel are prodrugs that require oxidation by the hepatic cytochrome P450-1A enzyme system to acquire activity (CYP2C19 for ticlopidine and CYP3A4 for clopidogrel), and in turn both drugs inhibit CYP2B6.62 Both are selective noncompetitive inhibitors of the P2Y12 receptor. The active metabolites of ticlopidine and clopidogrel induce a permanent defect that involves a single platelet-signaling pathway for the lifetime of the cell via cumulative inhibition at low doses in a manner similar to the pharmacodynamics of aspirin. Recent evidence indicates that P2Y12 receptors exist in homo-oligomeric complexes associated with platelet cell membrane lipid rafts and that the active metabolite of clopidogrel partitions the receptor out of the rafts to disrupt these oligomers, which thereby prevents signal transduction.63

Ticlopidine is rapidly absorbed and extensively metabolized, and onset of platelet inhibition (250 mg twice-daily PO) occurs within 24 to 48 hours with maximal effect at 3 to 5 days. Food enhances absorption, whereas antacids slow the process. Pharmacokinetic variability may reflect interindividual variation in metabolic clearance.64 Poor tolerance of larger loading doses (>500 mg) precludes this approach to achieve earlier platelet inhibition.62 Diarrhea, nausea, and vomiting are common side effects (30% to 50%). Skin rash is also a frequent problem. Neutropenia is reported in approximately 2% of recipients and has resulted in fatality.65 Because of these factors, ticlopidine use is now largely reserved for patients who are unable to tolerate clopidogrel.

Much of the clopidogrel dose undergoes esterase deactivation, and therefore only a small portion is metabolized to its active moiety in the liver. After hepatic metabolism, peak plasma metabolite concentrations occur at 1 hour, and bioavailability is unaffected by food.66 Ex vivo inhibition of platelet aggregation is dose- and time-dependent, and, in the absence of loading, a maximal effect (40% to 60% inhibition of ADP-induced aggregation ex vivo) occurs after 3 to 5 days. Platelet function recovers 3 to 5 days after drug withdrawal. With a loading dose of 300 mg clopidogrel, maximum inhibition of platelet aggregation occurs within 6 hours. However, full clinical benefit may not be achieved for 24 hours. Maximum antiplatelet response is attained approximately 2 hours after a loading dose of 600 mg, which is generally well tolerated and appears optimal.66

Clinical Trials With Clopidogrel
Clear benefit from ADP receptor blockade has been established in the secondary prevention of cardiovascular disease, independent of COX pathway inhibition.57 Furthermore, complementary mechanisms of action of aspirin and clopidogrel translate into additive benefit in certain populations (Table 3). An additive effect on bleeding time is also apparent. Particular consideration of risk versus benefit is therefore necessary when prolonged therapy in lower risk patients is considered.

Clopidogrel Resistance or Nonresponse
Variable Response to Clopidogrel
The concept of an incomplete clopidogrel response has arisen because multiple studies demonstrate interindividual variability in platelet response, and several small studies have associated an incomplete treatment response with recurrent cardiovascular events (Table 2). Furthermore, incomplete inhibition of ADP-induced platelet aggregation has been demonstrated in several studies of patients after stent thrombosis has occurred. It is unclear, however, if incomplete response to clopidogrel, aspirin, or both agents contributes to this complication (Table 2).
### TABLE 3. Randomized Clinical Trials of Clopidogrel Use to Treat Vascular Disease

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Subjects (n, % Female, Mean Age)</th>
<th>Treatment Arms</th>
<th>Mean Follow-Up Period</th>
<th>Primary End Point as Reported</th>
<th>RRR* (95% CI, P)</th>
<th>Event Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLARITY TIMI28 (2005)68</td>
<td>Acute STEMI (&lt;12 hours); Rx Lysis, ASA, ± heparin (3491, 19.7; 57 years)</td>
<td>Clopidogrel 300 mg bolus, 75 mg once daily vs placebo</td>
<td>3.5 d</td>
<td>Occluded infarct-related artery, death, MI prior to angiography (odds reduction of end point, 36%; 95% CI, 24 to 47; (P&lt;0.001))</td>
<td>30.9</td>
<td>15</td>
</tr>
<tr>
<td>PCI-CLARITY TIMI 28 (2005)69</td>
<td>Acute STEMI (&lt;12 hours); Rx Lysis, ASA, ± heparin, &amp; subsequent PCI (1863, 18.3; 57.3 years)</td>
<td>Clopidogrel 300 mg bolus, 75 mg once daily vs placebo and clopidogrel after angiogram</td>
<td>30 d</td>
<td>Cardiovascular death, MI, CVA from PCI to 30 days after randomization (adjusted OR, 0.54; 95% CI, 0.35 to 0.86; (P=0.008))</td>
<td>41.9</td>
<td>3.6</td>
</tr>
<tr>
<td>COMMIT (CCS-2) (2005)70</td>
<td>Suspected acute MI; Rx Lysis 54%, heparin (45 852, 29, 61 years)</td>
<td>Clopidogrel (no bolus) 75 mg once daily + ASA 162 mg vs placebo + ASA 162 mg once daily</td>
<td>Discharge date or 28 d</td>
<td>Death, reinfarction, CVA (all-cause mortality RRR, 7% [1 to 13]; (P=0.03))</td>
<td>9.0</td>
<td>9.2</td>
</tr>
<tr>
<td>CURE (2001)71</td>
<td>Acute coronary syndrome (12 562, 38.5%, 64.2 years)</td>
<td>Clopidogrel 300 mg bolus, 75 mg once daily + ASA 75 to 325 mg vs placebo + ASA 75 to 325 mg</td>
<td>9 m</td>
<td>Cardiovascular death, nonfatal MI, CVA (RR, 0.80; 95% CI, 0.72 to 0.90; (P&lt;0.001))</td>
<td>20.0</td>
<td>9.3</td>
</tr>
<tr>
<td>PCI-CURE (2001)72</td>
<td>Acute coronary syndrome on PCI (2658, 30.2%, 61.5 years)</td>
<td>Clopidogrel 300 mg bolus, 75 mg once daily + ASA 75 to 325 mg vs placebo + ASA 75 to 325 mg</td>
<td>8 m</td>
<td>Cardiovascular death, MI, urgent target vessel revascularization (&lt;30 days) (RR, 0.70; 95% CI, 0.50 to 0.97; (P=0.03))</td>
<td>30.0</td>
<td>4.5</td>
</tr>
<tr>
<td>CREDO (2002)73</td>
<td>Elective PCI or high risk likelihood of PCI (2116, 28.7%, 61.7 years)</td>
<td>Clopidogrel 300 mg bolus, 75 mg once daily + ASA 325 mg vs placebo + ASA 325 mg</td>
<td>1 y</td>
<td>Death, MI, CVA (3.9 to 44.4); (P=0.02)</td>
<td>26.9</td>
<td>8.5</td>
</tr>
<tr>
<td>CAPRIE (1996)74</td>
<td>Recent MI, CVA, or PAD (19 185, 28%, 62.5 years)</td>
<td>Clopidogrel 75 mg once daily vs ASA 325 mg</td>
<td>1.9 y</td>
<td>Ischemic stroke, MI, vascular vs ASA 325 mg death (P=0.043)</td>
<td>8.7</td>
<td>5.32</td>
</tr>
<tr>
<td>CHARISMA (2006)74</td>
<td>Clinically evident CAD or multiple risk factors (15 603, 29.8%, 64 years)</td>
<td>Clopidogrel 75 mg + ASA 75 to 162 mg vs placebo + ASA 75 to 162 mg</td>
<td>28 m†</td>
<td>Cardiovascular death, MI, CVA (RR, 0.93; 95% CI, 0.83 to 1.05; (P=0.22))</td>
<td>7.0</td>
<td>6.8</td>
</tr>
<tr>
<td>MATCH (2004)75</td>
<td>Recent ischemic CVA or TIA (7599, 37%, 66.3 years)</td>
<td>Clopidogrel 75 mg + ASA 75 mg once daily vs clopidogrel 75 mg + placebo</td>
<td>18 m</td>
<td>Ischemic CVA, MI, vascular death, or rehospitalization for acute ischemia (4.6 to 16.3); (P=0.24)</td>
<td>6.4</td>
<td>15.7</td>
</tr>
<tr>
<td>ACTIVE-W (2005)76</td>
<td>A fib and ≥1 stroke risk factor (6706, 23.5%, 70.2 years)</td>
<td>Clopidogrel 75 mg once daily and ASA 75 to 100 mg vs warfarin</td>
<td>1.28 y†</td>
<td>Stroke, non-central nervous system systemic embolism, MI, and vascular mortality (RR, 1.44; 95% CI, 1.18 to 1.76; (P=0.0003))</td>
<td>43.5</td>
<td>5.64</td>
</tr>
</tbody>
</table>

OR indicates odds ratio; RR, relative risk; RRR, relative risk reduction; and TIA, transient ischemic attack.

*Crude calculation from reported primary point event rate when not available in published results (95% CI provided when available).

†Median value.

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The definition of “clopidogrel resistance” and assay specifications vary from study to study. The predominant assay employed is ADP-induced platelet aggregation measured by light transmittance aggregometry. Nonstandardized methods, use of varying doses of ADP, and determination of either absolute difference, final, or maximum aggregation makes comparison of study results difficult. Furthermore, ADP-induced platelet aggregation is mediated by both P2Y1 and P2Y12, and the relative contribution of these receptors is known to vary between individuals and thus may confound assays of clopidogrel response. Flow cytometric evaluation of P-selectin, activated GP IIb/IIIa expression, or phosphorylated vasodilator-stimulated phosphoprotein are less widely available alternative assays (Table 2). In a manner similar to aspirin response, variable platelet response to clopidogrel probably represents a composite of processes, which include noncompliance, variable absorption, metabolism and receptor sensitivity, and enhanced baseline platelet reactivity.

**Dosing, Compliance, and Platelet Reactivity**

Studies indicate that the minimum daily dose of clopidogrel required to achieve optimal platelet inhibition is 60 mg, and most patients receive 75 mg clopidogrel daily. In contrast, daily dosing with 30 mg plain aspirin inhibits platelet COX in healthy volunteers. However, most patients are maintained on a dose at least 2-fold greater. Thus, the cumulative irreversible effect
expected during repeat daily dosing with clopidogrel may be undermined by even moderately poor compliance. Noncompliance with clopidogrel therapy may be a frequent problem and may be associated with significant morbidity and mortality.77

Others have detected a relationship between platelet aggregability at baseline and variability in clopidogrel response.78 Optimized dosing may partially attenuate this effect. However, platelets in unstable high-risk patients are simultaneously exposed to multiple agonists and lack redundancy in their signaling pathways, which may enhance baseline aggregability and modulate drug response.79 On-treatment platelet reactivity and response to single or combined antiplatelet therapy have been evaluated with several small studies demonstrating platelet reactivity, which correlated with cardiovascular morbidity.43

Healthy volunteers have variability in clopidogrel response and respond in a manner that is dose- and time-dependent.80 Patients post-PCI who take standard-dose clopidogrel (300 mg loading and 75 mg daily maintenance) also respond heterogeneously, and time-dependence of response indicates inadequate clopidogrel loading.81 Indeed, response to single-bolus clopidogrel is dose-related, and more rapid platelet inhibition is achieved with a higher loading dose (600 mg), which may be associated with improved outcomes in patients who undergo PCI.82

Clopidogrel response ex vivo assayed by platelet aggregometry forms a normal bell-curve distribution.83 However, unlike the profound antagonism detected in aspirin and GP IIb/IIIa receptor blocker assays, standard-dose clopidogrel (300 mg loading and 75 mg once-daily maintenance) achieves maximally 40% to 50% inhibition of ADP-induced platelet aggregation. Addition of a clopidogrel bolus during chronic clopidogrel therapy (75 mg per day) achieves additional platelet inhibition and may indicate the need for higher maintenance doses in some individuals.84 Indeed, the recent updated AHA guidelines for PCI provide for higher loading and maintenance doses in certain settings.

Pharmacogenetics
Pharmacodynamic heterogeneity occurs with most drugs to varying degrees. Genotypic variation is known to modulate platelet reactivity and thus may influence clopidogrel response. Several genetic mutations that modulate both P2Y12 function and expression have been identified.85,86 Furthermore, small studies of common sequence variation in the genes that encode the P2Y1 and P2Y12 receptor have detected an association with platelet response to ADP in vitro, predominantly at lower agonist concentrations.87 An effect on clopidogrel response, however, has not been discerned.88 Correlation between carriage of the human platelet alloantigen membrane GP IIIa variant (PLA2), and the antithrombotic effect of clopidogrel in patients with CAD has also been explored. However, data from these studies are conflicting.89,90

Pharmacokinetic Variability
Marked interindividual variability in clopidogrel pharmacokinetics has been confirmed after high loading doses. Differences in oral absorption, variable metabolism, failure to clear the active metabolite, and differing ADP receptor reactivity may each contribute. Evidence supports variable oral absorption as a prominent factor.91

Two of the more abundant CYP450 isozymes in the liver, CYP3A4 and CYP3A5, appear to metabolize clopidogrel most rapidly and are therefore credited with its transformation to the active metabolite. Indeed, a correlation between CYP3A4 activity and platelet inhibition by clopidogrel has been demonstrated.92 Existence of a clinically relevant interaction between clopidogrel and CYP3A4-metabolized statins is proposed, though this association is contentious and requires further evaluation.92,93 Relative substrate concentration and binding site affinity determine competitive inhibition. Clopidogrel is a reversible competitive inhibitor of CYP3A4. Therefore, potential for interaction exists particularly when lower clopidogrel doses coincide with higher statin doses. Furthermore, in vitro, clopidogrel metabolism is inhibited by >90% when clopidogrel and atorvastatin are present at equimolar concentrations.94

Alternative Adenosine Diphosphate Inhibitors
Additional P2Y12 receptor antagonists are under development and may provide more predictable levels of ADP inhibition. Prasugrel (CS-747, LY 640315) is an oral irreversible P2Y12 inhibitor that requires metabolism to acquire activity in a similar manner to clopidogrel. It is a more potent drug and achieves more rapid and consistent platelet inhibition.95 Cangrelor (AR-C69931MX) and AZD6140 are reversible and direct P2Y12 inhibitors. AZD6140 is administered orally, and Cangrelor is administered parenterally.96,97 Rapid onset and offset of platelet inhibition with Cangrelor makes its use attractive in the acute setting and as an adjunct to PCI. With the absence of a clinically relevant and desirable level of P2Y12 receptor inhibition and lessons learned regarding risk–benefit margins in thienopyridine trials, consistent rather than potent platelet inhibition over shorter durations may be a prudent initial goal.

Conclusion
Aspirin and clopidogrel provide significant benefit in patients with cardiovascular disease; however, evidence of variable platelet response has led to the concept of aspirin and clopidogrel “resistance.” Rather than true “resistance” to these antiplatelet agents, there is a variable response to aspirin and clopidogrel that reflects a variety of mechanisms.

Disclosures
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