Clinical Applications of Antiplatelet Therapy

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Dual antiplatelet therapy with aspirin and a thienopyridine has become the standard of care for patients undergoing percutaneous intervention with stenting, regardless of indication. This article will examine the evidence for and against the use of aspirin and thienopyridines, with emphasis on platelet resistance and nonresponsiveness. Data suggest that in some patients, clopidogrel plus aspirin is not superior to aspirin alone. Resistance to aspirin and clopidogrel has been reported. Patients exhibiting aspirin resistance, as measured by an elevated platelet aggregate ratio, have a 10-fold increase in the risk of recurrent vascular events as compared to aspirin-sensitive patients. Clopidogrel nonresponsiveness has been a consistently observed phenomenon in studies utilizing various $P2Y_{12}$ receptor–specific assays. Nonresponsiveness to clopidogrel treatment has been suggested as a risk factor for the occurrence of ischemic events and stent thrombosis.

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Platelet Activation

In the normal physiological state platelets are small, anucleate, subcellular fragments that circulate freely in the blood. However, disruption of the normal protective nature of the endothelium occurs during acute coronary syndromes and percutaneous coronary intervention (PCI), exposing the subendothelial matrix. Transient binding of platelet surface receptors to von Willebrand's factor and collagen present in the subendothelial matrix facilitates initial platelet adhesion to the vessel wall. Subsequent intracellular signaling events trigger platelet activation, granule secretion, and, finally, the activation of the glycoprotein

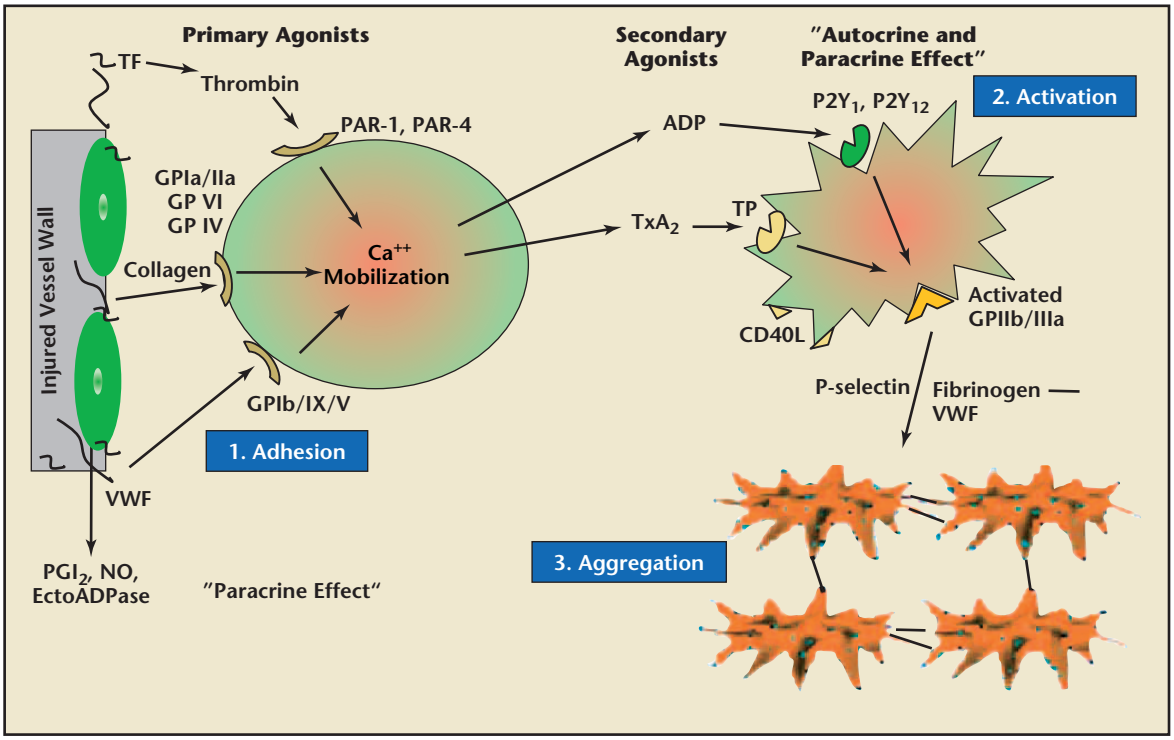
(GP) IIb/IIIa receptors. Binding of fibrinogen to the activated GPIIb/IIIa receptor facilitates irreversible platelet aggregation, further recruitment of platelets, and occlusive thrombus generation (platelet hypothesis)[1-3] (Figure 1).

In addition to von Willebrand's factor, thrombin is a primary agonist responsible for platelet activation. It is initially generated through tissue factor released from the damaged vessel wall and binds (mainly) to the protease activated receptor-1 (PAR-1).[4,5] Following activation, arachidonic acid (AA) is released from membrane phospholipids through phospholipase A_2 activity and then converted to thromboxane (Tx) A_2 by sequential actions of cyclooxygenase-1 and Tx synthase. Secreted TxA_2 binds to a specific Gq-coupled Tx receptor and activates surrounding platelets as a positive feedback mechanism.[1-3]

Adenosine diphosphate (ADP), a secondary agonist, is released from dense granules and activates surrounding platelets by binding to the specific G-protein–coupled purinergic receptors $P2Y_{12}$ and $P2Y_1$. The binding of ADP to the $P2Y_{12}$ receptor and subsequent activation of intracellular signaling pathways is primarily responsible for the activation of the GPIIb/IIIa receptor when primary agonists are at low concentrations. Thus, ADP and TxA_2 amplify platelet activation and the recruitment of platelets during stable thrombus generation. In addition, platelet activation results in the expression of surface adhesion molecules, especially P-selectin and CD40 ligand, leading to heterotypic aggregation of platelets with leukocytes. This heterotypic aggregation is important in inflammation and amplification of thrombin generation.[6,7]

Increased expression of platelet surface molecules and heightened platelet reactivity to agonists has been demonstrated in patients with acute coronary syndromes, diabetes, hypertension, hyperlipidemia, and during PCI.[8-10] Heightened platelet reactivity despite dual antiplatelet therapy with aspirin and clopidogrel has also been associated with stent thrombosis, restenosis, inflammation, myocardial infarction, and other ischemic events following stenting.[11-20] Autopsy studies have confirmed the primary involvement of platelets during thrombus formation in the setting of plaque rupture and plugging of the microvasculature in acute myocardial infarction.[21] Therefore, the rationale for antiplatelet therapy is to attenuate platelet activation (thereby attenuating the development of occlusive thrombus formation), to arrest platelet procoagulant activity, to

Figure 1. *Platelet adhesion, activation, and aggregation. TF, tissue factor; PAR, protease activated receptor; GP, glycoprotein; VWF, von Willebrand's factor; ADP, adenosine diphosphate; Tx, thromboxane; TP, thromboxane A_2 receptor. Data extracted from Gurbel PA et al.*[6] www.medreviews.com



inhibit platelet-mediated inflammation, to promote disaggregation of platelets, and, finally, to facilitate the reperfusion of occluded blood vessels.

Platelet Inhibition

Optimal platelet inhibition is dependent on the degree of ischemic risk and is counterbalanced by the risk of bleeding. Since platelet activation is a complex process involving multiple receptors and redundant pathways, the effective prevention of adverse events should focus on regimens that simultaneously inhibit multiple platelet activation pathways. Effective inhibition of the cyclooxygenase (COX) pathway by aspirin and the $P2Y_{12}$ receptors by thienopyridines is desirable during PCI and other high-risk states. Reversible, potent, predictable, and short-term platelet inhibition by GPIIb/IIIa receptor inhibitors is recommended during PCI in high-risk patients to prevent platelet-mediated thrombotic ischemic events.[22] Guidelines for antiplatelet therapy are listed in Table 1.

Aspirin

Aspirin binds to and irreversibly inactivates the active site of COX in platelets[23] through acetylation, resulting in the reduced production of TxA_2—a potent activator of platelets. Aspirin therapy is well established in primary and secondary prevention of cardiovascular events in several populations.[24] In primary prevention, the landmark Physicians' Health Study (PHS) was the first to report a benefit for aspirin (325 mg every other day) by showing a 44% reduction in first myocardial infarction (MI) among apparently healthy male physicians aged 40 to 84 years,[25] although there were insufficient events to determine the effects on cardiovascular death or stroke. These data have been subsequently confirmed in a meta-analysis of more than 50,000 subjects from 5 major trials, including PHS, which showed a 32% reduction in first MI and a 15% reduction in important vascular events, but no significant difference in stroke or vascular death.[26] The United States Preventive Services Task Force recommends the use of aspirin for primary prevention in individuals who are at increased risk for coronary heart disease.[27] Of recent interest is the publication of the Women's Health Study, in which more than 39,000 apparently healthy women were randomized to receive aspirin (100 mg every other day) or placebo and followed for a mean of 10 years. This trial showed no difference in the rate of first MI (RR, 1.02; 95% CI, 0.84-1.25) or cardiovascular death, although a 17% reduction in stroke was observed.[28] This benefit was offset in part by a small but significant increase in gastrointestinal bleeding. In the subgroup of women aged 65 or older, first MI was reduced by 32%, highlighting the importance of baseline risk in the determination of which patients should use aspirin for primary prevention.

The secondary prevention of cardiovascular events in patients with known atherosclerotic vascular disease with long-term aspirin therapy has been conclusively demonstrated. The Antithrombotic Trialists' Collaboration performed a meta-analysis of 195 trials with more than 130,000 subjects with prior evidence of cardiovascular disease (MI, stroke, peripheral vascular disease, atrial fibrillation) treated with antiplatelet therapy (predominantly aspirin) or placebo.[29] The analysis demonstrated a reduction in important vascular events by 22%. The Second International Study of Infarct Survival (ISIS-2) trial demonstrated a 23% reduction in vascular mortality in the setting of acute myocardial infarction, which was similar to the magnitude of benefit achieved using fibrinolytic therapy with streptokinase.[30]

Despite the abundance of evidence for the efficacy of aspirin across multiple groups and indications, the optimal dose remains the subject of some controversy. Analysis of clinical trials across a dose range of 75 mg to 325 mg[29,31,32] have shown similar efficacy and rates of bleeding.[29] However, an analysis from the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) study demonstrated an increased risk of bleeding and no difference in efficacy with higher aspirin doses.[33] There is theoretical evidence to suggest that the relative affinity for COX-1 versus COX-2 at lower doses may be more advantageous than the more balanced effects seen at higher doses.[34]

Additional controversy surrounds potential interactions between aspirin and angiotensin-converting enzyme (ACE) inhibitors, with concern that aspirin may attenuate the beneficial effects of ACE inhibition—although this association has not been substantiated by clinical trial data.[35] Recent concerns have been raised regarding inhibition of the efficacy of aspirin when it is coadministered with nonsteroidal anti-inflammatory drugs, due to competition for the active site on the COX enzyme.[36]

Thienopyridines/Dual Therapy

ADP plays a central role among the multiple mediators of platelet activation and aggregation. ADP binds to $P2Y_1$ and $P2Y_{12}$ receptors on the platelet surface. Each receptor plays a complementary role in platelet activation and aggregation, with $P2Y_1$ supporting shape change and early and transient platelet aggregation, and $P2Y_{12}$ activation resulting in sustained platelet activation and

Table 1
American College of Cardiology/American Heart Association Guidelines for Antiplatelet Therapy*

	Class I Recommendation	Class IIa Recommendation	Class IIb Recommendation
PCI			
Aspirin	• Patients already taking daily chronic aspirin therapy should take 75 to 325 mg of aspirin before the PCI procedure is performed (Level of Evidence A) • Patients not already taking daily chronic aspirin therapy should be given 300 to 325 mg of aspirin at least 2 hours and preferably 24 hours before the PCI procedure is performed (Level of Evidence C) • After PCI, in patients with neither aspirin resistance, allergy, nor increased risk of bleeding, aspirin 325 mg daily should be given for at least 1 month after bare-metal stent implantation, 3 months after sirolimus-eluting stent implantation, and 6 months after paclitaxel-eluting stent implantation, after which daily chronic aspirin use should be continued indefinitely at a dose of 75 mg to 162 mg (Level of Evidence B)		
Clopidogrel	• A loading dose of clopidogrel should be administered before PCI is performed (Level of Evidence A) • An oral loading dose of 300 mg administered at least 6 hours before the procedure has the best established evidence of efficacy (Level of Evidence B) • In patients who have undergone PCI, clopidogrel 75 mg daily should be given for at least 1 month after bare-metal stent implantation (unless the patient is at increased risk for bleeding, then it should be given for a minimum of 2 weeks), 3 months after sirolimus stent implantation, and 6 months after paclitaxel stent implantation, and ideally up to 12 months in patients who are not at high risk of bleeding (Level of Evidence B)	• For patients with an absolute contraindication to aspirin, it is reasonable to give a 300 mg loading dose of clopidogrel, administered at least 6 hours before PCI, and/or GPIIb/IIIa antagonists, administered at the time of PCI (Level of Evidence C) • When a loading dose of clopidogrel is administered, a regimen of greater than 300 mg is reasonable to achieve higher levels of antiplatelet activity more rapidly, but the efficacy and safety compared with a 300 mg loading dose are less established (Level of Evidence C) • It is reasonable that patients undergoing brachytherapy be given daily clopidogrel 75 mg indefinitely and daily aspirin 75 to 325 mg indefinitely unless there is significant risk for bleeding (Level of Evidence C)	• In patients in whom subacute thrombosis may be catastrophic or lethal (unprotected left main, bifurcating left main, or last patent coronary vessel), platelet aggregation studies may be considered and the dose of clopidogrel increased to 150 mg per day if less than 50% inhibition of platelet aggregation is demonstrated (Level of Evidence C)
Unstable Angina or Non-STEMI			
Aspirin	• Aspirin should be administered as soon as possible after presentation and continued indefinitely (Level of Evidence A)		
Clopidogrel	• Clopidogrel should be administered to patients who are unable to take aspirin because of hypersensitivity or major gastrointestinal intolerance (Level of Evidence A) • In hospitalized patients in whom an early noninterventional approach is planned, clopidogrel should be added to aspirin as soon as possible on admission and administered for at least 1 month (Level of Evidence A) and for up to 9 months (Level of Evidence B) • In patients for whom a PCI is planned, see PCI guidelines, above		

*Class I: Conditions for which there is evidence for and/or general agreement that a given procedure or treatment is beneficial, useful, and effective; Class II: Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment; Class IIa: Weight of evidence/opinion is in favor of usefulness/efficacy; Class IIb: Usefulness/efficacy is less well established by evidence/opinion; Class III: Conditions for which there is evidence and/or general agreement that a procedure/treatment is not useful/effective and in some cases may be harmful; Level of Evidence A: Data derived from multiple randomized clinical trials or meta-analyses; Level of Evidence B: Data derived from a single randomized trial or nonrandomized studies; Level of Evidence C: Only consensus opinion of experts, case studies, or standard-of-care.

PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction; GP, glycoprotein. Data extracted from Smith SC Jr et al,[22] Antman EM et al,[44] and Braunwald E et al.[97]

aggregation.[37] Ticlopidine and clopidogrel are thienopyridine antiplatelet agents that irreversibly inhibit the $P2Y_{12}$ receptor. Dual antiplatelet therapy with aspirin and ticlopidine is superior to oral anticoagulation therapy, such as Coumadin,[38-40] in patients undergoing PCI with stent implantation. Therefore, dual antiplatelet therapy with a thienopyridine and aspirin has become the standard of care for patients undergoing PCI with stenting, regardless of indication.[22] Clopidogrel has marginally better efficacy[41] for the reduction of ischemic events, and it has largely replaced ticlopidine due to a better tolerability profile, especially for hematologic side effects.[42]

In a meta-analysis of 287 studies involving 135,000 patients considered to be at high annual risk of vascular events because of pre-existing disease or risk factors, antiplatelet therapy reduced serious vascular events by about 25%.[29] Other large-scale studies of antiplatelet agents have again recommended the broader use of these agents for the prevention of myocardial infarction, stroke, and vascular death.[43,44] The Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial demonstrated that clopidogrel alone was modestly superior to aspirin alone in the prevention of stroke, myocardial infarction, and vascular death in patients with a previous diagnosis of ischemic stroke, myocardial infarction, or symptomatic atherosclerotic peripheral arterial disease.[45] Greater benefits have been observed with dual antiplatelet therapy (aspirin plus clopidogrel) as compared to aspirin monotherapy in patients with unstable acute coronary syndromes, such as unstable angina/non–ST-segment elevation MI (NSTEMI), whether they were treated medically, surgically, or with PCI.[46-48] More recently, significant benefits have been observed with the addition of clopidogrel to aspirin among patients with ST-segment elevation MI (STEMI) treated with fibrinolytic therapy. In the Clopidogrel as Adjunctive Reperfusion Therapy-Thrombolysis In Myocardial Infarction 28 (CLARITY-TIMI 28) and the ClOpidogrel and Metoprolol in Myocardial Infarction Trial/Second Chinese Cardiac Study (COMMIT-CCS 2) trials, benefits were seen for improvement in coronary artery flow, reduction in myocardial infarction, and overall mortality with the addition of clopidogrel.[49,50]

Recent data from the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) trial suggest a parallel between primary and secondary prevention data with aspirin alone.[51] In patients with a history or high risk of coronary artery disease (CAD), clopidogrel plus aspirin was not superior to aspirin alone. A significant benefit of clopidogrel and aspirin, however, was seen in the highest risk patients (those with symptomatic cardiovascular disease) but not in asymptomatic patients with multiple risk factors. The use of clopidogrel in addition to aspirin as a primary prevention strategy was associated with an increased risk of bleeding and had no extra clinical benefits over aspirin therapy alone. The recent Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events-W (ACTIVE W) trial indicated that oral anticoagulation therapy was clearly superior to dual antiplatelet therapy with clopidogrel and aspirin for the prevention of vascular events in patients with atrial fibrillation who were at high risk of stroke.[52] These trials highlight the importance of understanding the pathophysiology of the disease state and of targeting dual antiplatelet therapy to patients at high risk for arterial thrombotic events.

Although clopidogrel has shown benefits across multiple indications its limitations include slow onset of antiplatelet effect, irreversibility of antiplatelet effect, and variability of antiplatelet response. Subsequent analysis from the Clopidogrel for the Reduction of Events During Observation (CREDO) trial showed that a period of pre-loading prior to PCI of at least 15 hours was necessary for reduction of major adverse cardiovascular events.[53] Thienopyridines bind irreversibly to the platelet $P2Y_{12}$ receptor, and reversal of the antiplatelet effect requires formation of new platelets, which takes up to 5 to 7 days. This feature has resulted in the hesitance of some operators to give clopidogrel to patients with planned PCI until the coronary anatomy is known, because of the increased risks of bleeding associated with subsequent coronary artery bypass surgery in patients who have received clopidogrel.[54-56] According to the American College of Cardiology/American Heart Association (ACC/AHA) STEMI and Unstable angina/NSTEMI guidelines, clopidogrel should be withheld for at least 5 days and preferably for 7 days in patients in whom coronary artery bypass grafting is planned, unless the urgency for revascularization outweighs the risks of excess bleeding (Level of Evidence B). This delayed efficacy combined with irreversible effects creates a conundrum for the clinician: early treatment is needed for benefit, but it may delay surgical therapy. In addition to these limitations, a variability of response to clopidogrel has been noted in several groups of patients, with some exhibiting inadequate antiplatelet effects following treatment with clopidogrel. The pharmacologic profile of available antiplatelet therapies is provided in Table 2.

Table 2
Pharmacologic Profile of Available Antiplatelet Therapies

	Aspirin	Clopidogrel	Ticlopidine
Efficacy	Established, guideline recommended	Established, guideline recommended	Not well established; clopidogrel preferred because of body of evidence and adverse event profile
Safety/Adverse Effects	Symptomatic GI disturbances in 2% to 10% of individuals; hypersensitivity 0.3%	Tolerability similar to aspirin; in CAPRIE, clopidogrel associated with less GI bleeding than aspirin (0.49% vs 0.71%); higher bleeding risk in elderly; neutropenia risk lower than ticlopidine (0.04%); TTP reported rarely	Severe neutropenia 0.8% to 1%
Dosing	75 mg to 325 mg at least 2 hours and preferably 24 hours prior to procedure, followed by 325 mg/d*	300 mg at least 6 hours pre-procedure, followed by 75 mg/d*	Not recommended
Resistance	≤ 1% to 54.7%	15% to 63% depending on time point	
Drug Interactions	NSAIDs, COX-2 inhibitors, salicylates, ACE inhibitors, alendronate, probenecid, valproic acid, many others	Activation thought to be mediated by CYP3A4; inhibitors of CYP3A4 may decrease its effect (amiodarone, protease inhibitors, azole antifungals, clarithromycin, erythromycin, fluoxetine, and others); inducers of CYP3A4 may increase its effect (rifampin, St. John's wort,[98] rifabutin, bosentan, carbamazepine, barbiturates); clopidogrel inhibits CYP2C9 and may increase plasma concentrations of drugs metabolized by this pathway (warfarin, NSAIDs, phenytoin, fosphenytoin, torsemide, tolbutamide). The clinical significance of the 2C9 interaction is unknown. Clopidogrel inhibits CYP2B6, and may affect drugs metabolized by this pathway. Drugs including bupropion and cyclophosphamide are metabolized to active forms by this pathway; thus concomitant administration of clopidogrel could lead to decreased effectiveness. The clearance of other drugs such as selegiline, ketamine, propofol, nevirapine, and efavirenz could also be affected[99]	
Cost Effectiveness	Inexpensive	Shown to be cost-effective in a variety of studies[100]	
Limitations	Onset and duration of effect, resistance, variability	Onset and duration of effect, resistance, variability	Side effect profile

*See Table 1 for more details on dose and treatment duration recommendations according to stent type.
GI, gastrointestinal; CAPRIE, Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events; TTP, thrombocytopenic purpura; NSAIDs, nonsteroidal anti-inflammatory drugs; COX, cyclooxygenase; ACE, angiotensin-converting enzymes; CYP, hepatic cytochrome P450.

Platelet Resistance and Nonresponsiveness

Both distinct response variability and nonresponsiveness have been demonstrated with aspirin and clopidogrel therapy. In addition, adverse clinical events have been associated with nonresponsiveness to antiplatelet therapy. Based on these factors, a closer look at the utility of these antiplatelet agents in different settings of vascular diseases is warranted. No single receptor signaling pathway mediating platelet activation is responsible for all thrombotic complications. Therefore, a single treatment strategy directed against a specific receptor cannot overcome all thrombotic complications. With this in mind, it is our opinion that the optimal definition of resistance or nonresponsiveness to an antiplatelet agent is the failure of the antiplatelet agent to inhibit the target of its action. The active metabolite of clopidogrel irreversibly inhibits the $P2Y_{12}$ receptor by forming a covalent disulfide bond.[57] Aspirin inhibits COX-1 by irreversibly acetylating the 529 serine residue.[58] The identification of resistance would therefore utilize a laboratory technique that detects residual activity of the target. In the case of aspirin, there would be residual post-treatment COX-1 activity; in the case of clopidogrel resistance, there would be evidence of residual post-treatment $P2Y_{12}$ activity. Because thrombosis involves multiple signaling pathways, treatment failure is not synonymous with drug resistance (Figure 2).

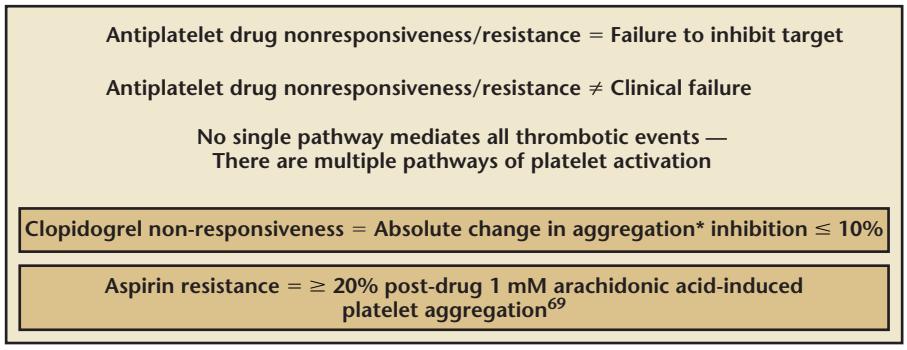


Figure 2. *Since thrombosis involves multiple signaling pathways, treatment failure is not synonymous with drug resistance. *Absolute change in aggregation is defined as maximum baseline aggregation minus maximum post-drug aggregation. This definition of clopidogrel nonresponsiveness is one of several. Data extracted from Tantry US, et al[70] and Gurbel PA, et al.[81]* www.medreviews.com

Aspirin

In 1978, Mehta and colleagues[59] reported that approximately 30% of patients with CAD had minimal inhibition of platelet function (unchanged bleeding time) with aspirin therapy. Since then, numerous studies have evaluated the efficacy of aspirin therapy using various laboratory methodologies to demonstrate the phenomenon of aspirin "resistance."[3] However, a reliable and specific laboratory method to identify aspirin resistance has not yet been uniformly accepted by investigators.

Use of various COX-1–specific and COX-1–nonspecific methods has shown the prevalence of aspirin resistance to be between ≤ 1% to 54.7% in different settings of cardiovascular disease (Table 3).[60-73] Available laboratory methods for assessing platelet activity can be categorized as COX-1 specific and COX-1 nonspecific. COX-1–specific methods include arachidonic acid–induced platelet aggregation by light transmittance assay using platelet-rich plasma or by thrombelastography with whole blood; enzyme-linked immunoassay determination of stable metabolites of TxA_2 and 11-dehydo TxB_2, especially in platelet-rich plasma after stimulation with AA or in urine; and the VerifyNow® point-of-care assay with AA as the agonist. COX-1–nonspecific methods include adenosine diphosphate-induced or collagen-induced platelet aggregation by light transmittance assay and the Platelet Function Analyzer (PFA)-100® (Dade-Behring, Miami, FL). Therefore, methods such as ADP-, epinephrine-, or collagen-induced platelet aggregation do not solely reflect COX-1 activity—the target of aspirin—and thus are fundamentally flawed in specifically measuring the platelet response to aspirin (Figure 3). The measured prevalence of aspirin resistance is usually higher based on these methods.

Prevalence of Aspirin Resistance

Use of laboratory techniques that reflect platelet COX-1 activity has demonstrated that aspirin resistance is rare (< 1%) among patients undergoing elective PCI who are treated with 325 mg/d aspirin. Nonadherence to therapy is a major factor (3% to 9%) contributing to the overestimation of aspirin resistance.[70,71] In a recent prospective, randomized, double-blind, double crossover investigation of aspirin dosing in patients with stable coronary artery disease, aspirin resistance was 1% to 5% when COX-1–specific methods were utilized and up to 30% with COX-1–nonspecific methods.[72,73] Moreover, there was no consistency in the measurement of aspirin responsiveness between point of service assays

Table 3
Aspirin Resistance Studies

Investigators	N	Patient Population	Aspirin Dose (daily)	Time	Method	Criteria for Aspirin Resistance	% Aspirin Resistance
Hurlen M et al[60]	143	Acute myocardial infarction	75 mg to 160 mg	2 hours to 24 hours	PAR	PAR < 0.82 PAR < 0.82 after additional aspirin	9.8 1.4
Buchanan MR and Brister SJ[61]	40	CABG	325 mg	?	Bleeding time platelet TxA_2, 12-HETE, and platelet adhesion	No prolongation of bleeding time above baseline	43
Buchanan MR et al[62]	287	CABG	325 mg	24 hours 2 years follow-up	Bleeding time	No prolongation of bleeding time above baseline	54.7
Grotemeyer KH et al[63]	180	Stroke	1500 mg	1 year follow-up	PR	Normal PR index (> 1.25) at 2 or 12 hours	33.3
Gum PA et al[64,65]	325	Stable CAD	325 mg	≥ 7 days 2 years follow-up	LTA-AA and ADP PFA-100 collagen/ ADP or collagen/EPI	> 70% ADP-induced aggregation + AA (0.5 mg/mL)-induced > 20% after ASA Normal (< 193 seconds) collagen/EPI closure time after aspirin	5.5 9.5
Eikelboom JW et al[66] (HOPE Study)	488	High risk of cardio-vascular events	75 mg to 325 mg	5 years follow-up	Urinary 11-dehydro TxA_2	Elevated urinary 11-dehydro TxA_2-upper quartile	25
Wang JC et al[67]	422	Stable CAD	81 mg to 325 mg	> 7 days	RPFA	ARU > 550	23
Chen W-H et al[68]	151	Non-urgent PCI	81 mg to 325 mg	> 7 days	RPFA Myonecrosis (CK-MB + TnI)	ARU > 550	19.2
Mehta SS et al[69]	203	Diabetes	325 mg	2 hours to 30 hours	RPFA	ARU > 550	18.7
Tantry US et al[70]	223	PCI	325 mg	Long-term	LTA-1 mM AA TEG	> 20% aggregation (LTA) > 50% aggregation (TEG)	< 1.0
Schwartz KA et al[71]	190	History of myocardial infarction	325 mg	≥ 1 month	LTA, Plateletworks	> 20% Aggregation (AA)	< 1.0
Gurbel PA et al[72]	100	Stable CAD	81, 162, and 325 mg	4 weeks each	LTA, VerifyNow (AA), TEG	> 20 aggregation (LTA), > 550 ARU (VerifyNow), > 50% aggregation (TEG)	≤ 1 (LTA) ≤ 5 (Verify Now) ≤ 5 (TEG)
Tantry U et al[73]	100	Stable CAD	81, 162, and 325 mg	4 weeks each	LTA (Collagen, ADP), PFA-100	> 70% aggregation (LTA) < 190 secs (PFA-100)	≤ 16 (LTA) ≤ 30 (PFA-100)

PAR, platelet aggregate ratio; CABG, coronary artery bypass graft surgery; HETE, hydroxyeicosenoic acid; PR, platelet reactivity; CAD, coronary artery disease; LTA, light transmittance aggregometry; AA, arachidonic acid; ADP, adenosine diphosphate; PFA, Platelet Function Analyzer; EPI, epinephrine; HOPE, Heart Outcomes Prevention Trial; RPFA, rapid platelet function analyzer; ARU, aspirin reaction units; PCI, percutaneous coronary interventions; TEG, thrombelastography; CK-MB, creatinine kinase-MB.

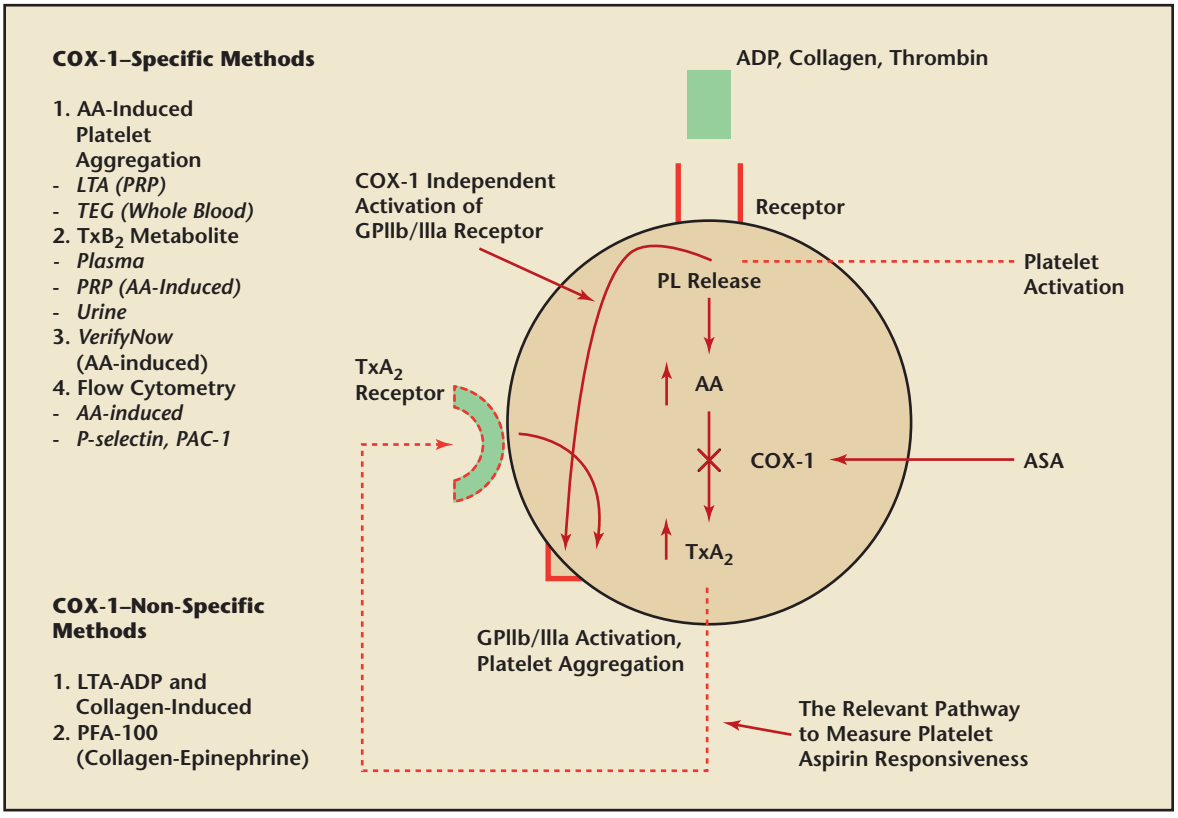


Figure 3. *Mechanism of action of aspirin and laboratory evaluation of aspirin nonresponsiveness. ADP, adenosine diphosphate; COX-1, cyclooxygenase-1; GP, glycoprotein; PL, phospholipids; Tx, thromboxane; AA, arachidonic acid; ASA, aspirin; LTA, light transmittance aggregometry; TEG, thrombelastography; PRP, platelet-rich plasma; PFA, platelet function analyzer.* www.medreviews.com

in patients with stable cardiovascular disease receiving different doses of aspirin. Thus, these studies indicate that noncompliance, COX-1 nonspecific methods, and, less importantly, underdosing may be factors responsible for the reported variability in aspirin resistance estimates in various clinical studies.

It has also been demonstrated in recent studies that aspirin resistance may be associated with clopidogrel resistance.[74,75] Moreover, aspirin-resistant patients exhibited high platelet reactivity induced by various agonists, such as collagen and ADP, in addition to arachidonic acid.[74-76] Therefore, aspirin resistance as measured in these studies may define a high platelet reactivity phenotype indicative of high risk for ischemic events.

Clinical Relevance of Aspirin Resistance

Grotemeyer and colleagues[63] found that stroke patients exhibiting aspirin resistance, as measured by an elevated platelet aggregate ratio, had a 10-fold increase in the risk of recurrent vascular events as compared to aspirin-sensitive patients (40% versus 4.4%; $P < .0001$). Using whole blood aggregometry to evaluate aspirin resistance, Mueller and colleagues[77] found an 87% increase in the incidence of reocclusion in "aspirin-resistant" patients who underwent balloon angioplasty and were treated with 100 mg/d of aspirin for 18 months. Gum and coworkers[64,65] found a 5% incidence of aspirin resistance based on the criteria of ≥ 20% AA-induced aggregation and ≥ 70% ADP-induced aggregation in patients with stable cardiovascular disease treated with 325 mg/d aspirin for up to 2.5 years. Aspirin resistance was associated with a significant increase in the composite endpoint of death, MI, or stroke.[64,65] Using a more COX-1–specific method measuring urinary Tx metabolite levels among patients enrolled in the Heart Outcomes Prevention Evaluation (HOPE) trial for aspirin responsiveness, Eikelboom and colleagues[66] found that the risk of MI, stroke, or cardiovascular death was greater in patients in the highest

quartile of urinary TxB_2 levels. These data suggest that incomplete suppression of TxA_2 production during aspirin therapy is a cardiovascular risk factor. Recently, Chen and coworkers,[68] using the Ultegra Rapid Platelet Function Assay-ASA (Accumetrics, San Diego, CA) with cationic propyl gallate as the agonist, found that despite treatment with clopidogrel and heparin, aspirin resistance as defined by this assay was associated with an increase of about 2.9-fold in the occurrence of myonecrosis following PCI.

Increased AA metabolism and TxA_2 synthesis may be responsible for enhanced platelet activation and reactivity observed in diabetic patients.[78] In addition, it was also suggested that diabetic patients exhibit subdued responsiveness to aspirin therapy.[79] Recently, researchers using the VerifyNow® Ultegra Rapid Platelet Function Assay-ASA, with cationic propyl gallate as an agonist, identified 18.7% resistance in diabetic patients who were taking a single dose of 325 mg aspirin.[69]

Clopidogrel

A standardized laboratory method that simulates the in vivo platelet response to antiplatelet therapy is still lacking. Since clopidogrel specifically inhibits 1 of 2 ADP receptors, ex vivo measurement of ADP-induced maximum platelet aggregation by light transmittance aggregometry has been the most commonly used laboratory method to evaluate clopidogrel responsiveness and is considered the gold standard. Recently, it was suggested that because antiplatelet drugs (especially clopidogrel) also induce platelet disaggregation, the response to clopidogrel would be better demonstrated by measuring late platelet aggregation at 6 minutes after stimulation with ADP rather than with measures of maximum aggregation.[80] However, unpublished data from the laboratory of Dr. Gurbel and Dr. Tantry, based on the evaluation of both maximum and final aggregation, indicated that both measurements were equivalent in determining the prevalence of clopidogrel nonresponsiveness. Flow cytometric measurements of the expression of the activated GPIIb/IIIa receptor and of P-selectin after ADP stimulation can also identify clopidogrel nonresponsiveness and correlate it with measurements of maximum aggregation stimulated by ADP.[16,17,81,82] Point of care assays can be used to measure clopidogrel responsiveness. Two such methods include whole blood thrombelastography, which measures ADP-induced platelet-fibrin clot strength, and the VerifyNow® $P2Y_{12}$ receptor assay, which uses ADP as an agonist of platelet activation.[11,83] The PFA-100® method using collagen-ADP–based cartridges and whole blood aggregometry are associated with inconsistent estimates of platelet reactivity to ADP. The phosphorylation state of vasodilator-stimulated phosphoprotein is a specific intracellular marker of residual $P2Y_{12}$ receptor reactivity in patients treated with clopidogrel and can be measured by flow cytometry. This technique is perhaps the most specific indicator of residual $P2Y_{12}$ activity in patients treated with a $P2Y_{12}$ inhibitor. However, the methodology is labor intensive and requires permeation of the platelet membrane and use of monoclonal antibodies specific for phosphorylated vasodilator-stimulated phosphoprotein[11,84] (Figure 4).

Prevalence of Clopidogrel Resistance

The phenomenon of clopidogrel response variability and nonresponsiveness was observed by measuring platelet aggregation in patients undergoing coronary stenting who were treated with dual antiplatelet therapy.[81,85] Marked response variability to clopidogrel treatment (300 mg loading dose followed by 75 mg/d maintenance dose) following stent implantation was demonstrated by light transmittance aggregometry and flow cytometric measurements of activation-dependent markers following stimulation with ADP. Clopidogrel resistance was defined as $\leq$ 10% absolute change in platelet aggregation from baseline levels. In this study, 63% of patients were resistant to clopidogrel treatment at 2 hours, 30% were resistant at day 1 and day 5 post-stenting, and 15% were resistant at day 30 (Figure 5). Therefore, clopidogrel "resistance" in this study appeared to be time dependent. The phenomenon of clopidogrel "resistance" has been confirmed by multiple investigators in patients undergoing stenting and is now variously described as "nonresponsiveness," "hyporesponsiveness," or "clopidogrel resistance" (Table 4).[86-93]

Clopidogrel nonresponsiveness is also dependent on dose. In a pharmacodynamic study comparing 300-mg and 600-mg clopidogrel loading doses, treatment with the 600-mg loading dose during PCI reduced clopidogrel nonresponsiveness to 8% compared to 28% to 32% with the 300-mg loading dose (Figure 6).[85] Moreover, this study demonstrated a narrower response profile following treatment with 600 mg of clopidogrel. These data suggest that thienopyridines with greater potency may be associated with a lower rate of nonresponsiveness. Similar increased platelet inhibition and decreased clopidogrel nonresponsiveness following a 600-mg loading dose has been observed in another clinical evaluation.[87] Patient demographics may also play a role in the response to clopidogrel

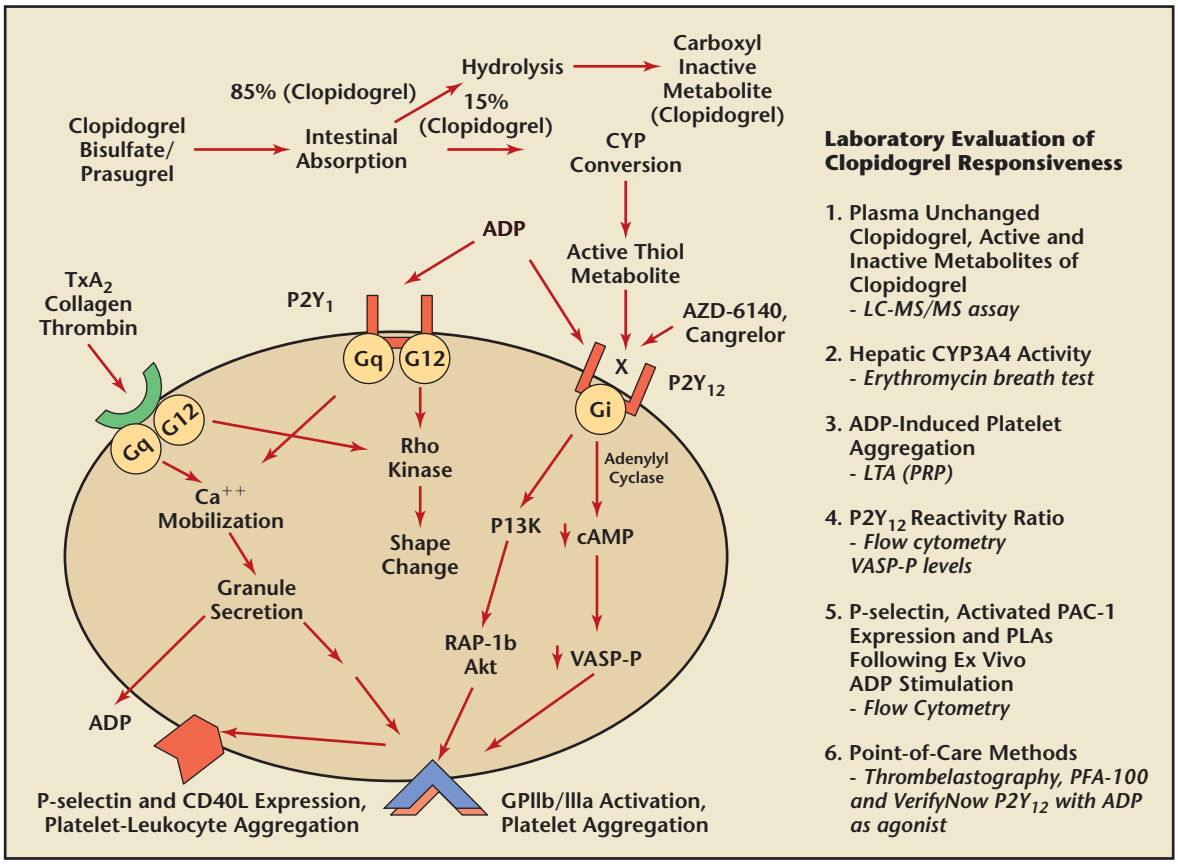


Figure 4. *Mechanism of action of clopidogrel and laboratory evaluation of clopidogrel nonresponsiveness. CYP, hepatic cytochrome P450; ADP, adenosine diphosphate; Tx, thromboxane; cAMP, cyclic adenosine monophosphate; VASP-P, vasodilator stimulated phosphoprotein-phosphorylated; GP, glycoprotein; LC-MS/MS, liquid chromatography-mass spectrometry; LTA, light transmittance aggregometry; PRP, platelet-rich plasma; PLA, platelet-leukocyte aggregation; PFA, Platelet Function Analyzer.* www.medreviews.com

therapy. In a recent study diabetic patients who were on long-term dual antiplatelet therapy were more likely to be clopidogrel nonresponders than nondiabetic patients.[93]

Clinical Relevance of Clopidogrel Resistance

Limited data are available to link clopidogrel nonresponsiveness to the occurrence of thrombotic events. Matetzky and colleagues[12] studied clopidogrel responsiveness in patients undergoing stenting for acute ST-elevation MI and found that patients who exhibited the highest quartile of ADP-induced aggregation had a 40% probability for a recurrent cardiovascular event within 6 months. In the Platelet REactivity in Patients And Recurrent Events (PREPARE) Post-Stenting study, patients suffering a recurrent ischemic event within 6 months of elective stenting had high post-stent platelet reactivity to ADP compared to patients without ischemic events, despite dual antiplatelet therapy.[18] In the Clopidogrel Loading with Eptifibatide to Arrest PLATELET reactivity Study (CLEAR PLATELETS) and CLEAR PLATELETS Ib, a 600-mg clopidogrel loading dose used to treat patients undergoing elective stenting was associated with superior early platelet inhibition compared to a 300-mg loading dose; the superior platelet inhibition was sustained over 24 hours. In turn, increased platelet inhibition was accompanied by a decrease in release of myocardial necrosis and inflammation markers.[16,17] Cuisset and colleagues[20] demonstrated that patients with high post-treatment platelet reactivity had an increased risk of cardiovascular events. More importantly, these patients were resistant to both clopidogrel and aspirin treatment. Similarly, Lev and colleagues[74] demonstrated that occurrence of a creatinine kinase-myocardial band after stenting was more frequent in patients exhibiting aspirin and clopidogrel resistance. Finally, a significantly higher rate of recurrent ischemic events within 6 months of elective coronary stenting

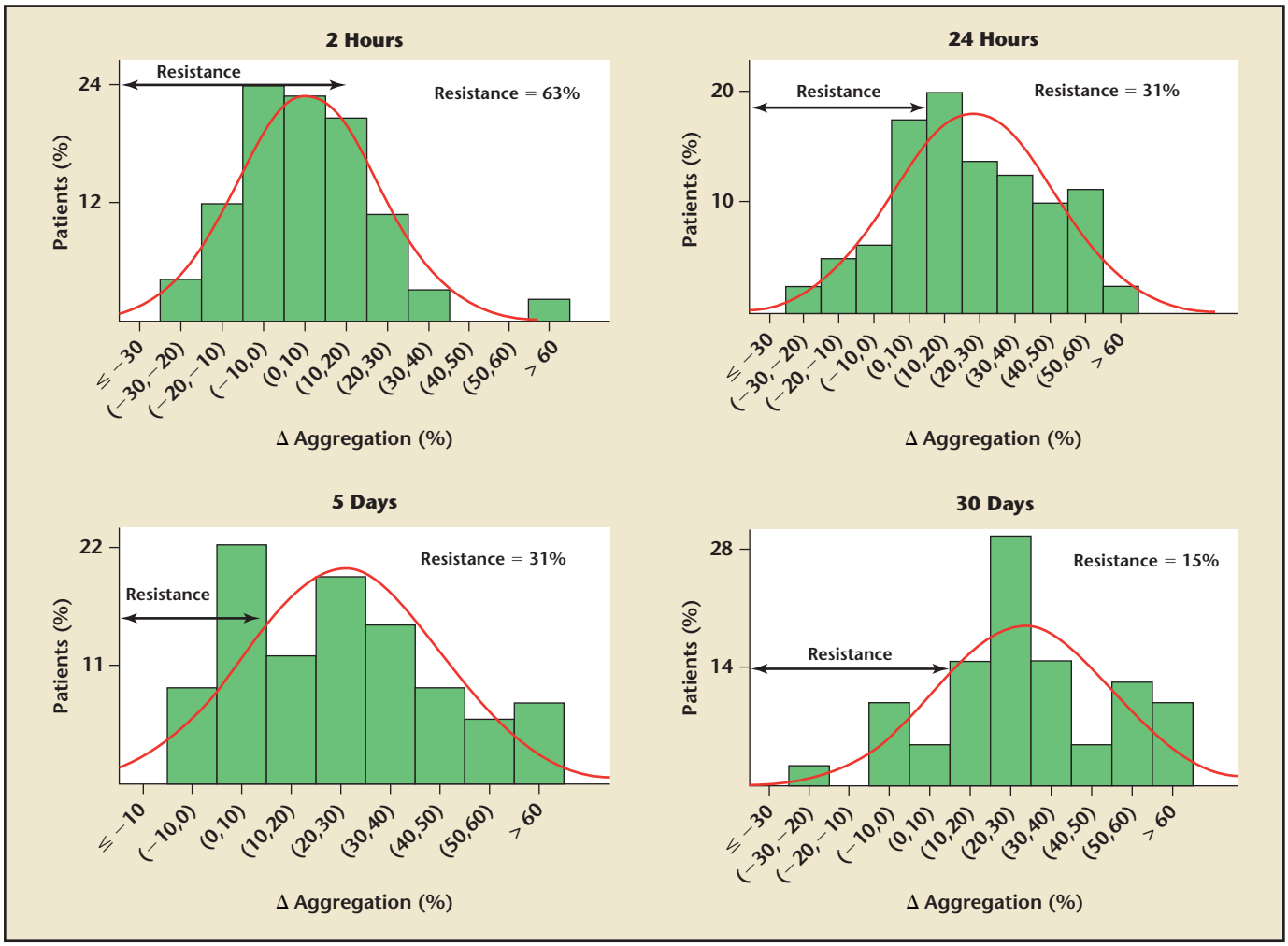


Figure 5. *Relationship between frequency of patients and absolute change in aggregation (Δ Aggregation [%]) in response to 5 μM ADP at 2 hours, 24 hours, 5 days, and 30 days after stenting. Δ Aggregation (%) is defined as baseline aggregation (%) minus post-treatment aggregation (%). Resistance, as defined herein, is Δ Aggregation ≤ 10%. Resistance is present in those patients subtended by the double-headed arrow. Curves represent normal distribution of data. Adapted with permission from Gurbel PA et al.*[81] www.medreviews.com

was observed in patients who were on chronic clopidogrel therapy and who had exhibited higher pre-procedure ADP-induced platelet aggregation.[19] All of these findings strongly suggest that in patients who are currently taking recommended antiplatelet therapy, high platelet reactivity is a risk factor for ischemia associated with PCI.

Based on the analysis of flow cytometric measurements of intracellular VASP phosphorylation levels, nonresponsiveness to clopidogrel treatment has been suggested as a risk factor for the occurrence of stent thrombosis.[11,94] In the recent Clopidogrel effect on platelet REactivity in patients with Stent Thrombosis (CREST) study, elevated levels of ADP-induced platelet aggregation, ADP-stimulated expression of active GPIIb/IIIa expression, and the $P2Y_{12}$ reactivity ratio measured by VASP phosphorylation were observed in patients with stent thrombosis compared to patients without stent thrombosis, indicating inadequate inhibition of the $P2Y_{12}$ receptor.[11] Other investigators have reported that high ex vivo shear-induced platelet aggregation despite dual antiplatelet therapy may be a risk factor for stent thrombosis.[13]

The Optimal Oral Antiplatelet Agent

Given the significant success of aspirin, thienopyridines, and dual antiplatelet therapy, what improvements could be made on the existing standard? Advances in the efficacy,

Table 4
Clopidogrel Resistance Studies

Investigators	N	Patient Population	Clopidogrel Dose (load/qd)	Definition of Clopidogrel Resistance	Time	Incidences
Gurbel PA et al[81]	92	PCI	300 mg/75 mg	5 μM and 20 μM ADP-induced aggregation < 10% absolute change	24 hours	31%-35%
Jaremo P et al[86]	18	PCI	300 mg/75 mg	ADP-induced fibrinogen binding < 40% of baseline	24 hours	28%
Muller I et al[87]	119	PCI	600 mg/75 mg	5 μM and 50 μM ADP-induced aggregation < 10% relative change	4 hours	5%-11%
Mobley JE et al[89]	50	PCI	300 mg/75 mg	1 μM ADP-induced aggregation, TEG and Ichor PW; < 10% absolute inhibition	Before and after clopidogrel therapy	30%
Lepantalo A et al[90]	50	PCI	300 mg/75 mg	2 μM or 5 μM ADP-induced aggregation and PFA-100 10% inhibition and 170 seconds	2.5 hours	40%
Angiolillo DJ et al[91]	48	PCI	300 mg/75 mg	6 μM ADP-induced aggregation < 40% inhibition	10 minutes, 4 hours, and 24 hours	44%
Matetzky S et al[12]	60	STEMI	300 mg/75 mg	5 μM ADP-induced aggregation and CPA < 10% inhibition	Daily for 5 days	25%
Serebruany VL et al[92]	544	Heterogeneous population	300 mg/no loading dose	5 μM ADP-induced aggregation 2 standard deviations below the mean	Up to 30 days	4.2%
Gurbel PA et al[85]	190	PCI	300 mg or 600 mg/75 mg	5 μM and 20 μM ADP-induced aggregation < 10% absolute inhibition	24 hours	28%-32% with 300 mg 8% with 600 mg
Angiolillo DJ et al[93]	52	Diabetic and non-diabetic	300 mg	< 10% relative inhibition	24 hours	38% diabetics 8% non-diabetics
Lev EI et al[74]	150	PCI	300 mg	5 μM ADP-induced aggregation < 10% absolute change	24 hours	24%

PCI, percutaneous coronary interventions; ADP, adenosine diphosphate; TEG, thrombelastography; PW, Plateletworks; PFA, Platelet Function Analyzer; STEMI, ST-segment elevation myocardial infarction; CPA, cone and platelet analyzer.

safety, and/or convenience of antiplatelet therapy would be an important contribution to the care of patients with cardiovascular disease. In terms of efficacy, agents that have rapid onset may allow for reduction of cardiovascular events in a shorter time period, especially when coronary intervention is performed on an urgent basis—when extended pretreatment may not be possible. The optimal agent or combination of agents should block both activation and aggregation of platelets to multiple stimuli. Although the optimal level of inhibition is not known, outcomes are likely to be improved by more potent agents that can achieve higher levels of inhibition, with greater consistency of response, than those achieved with

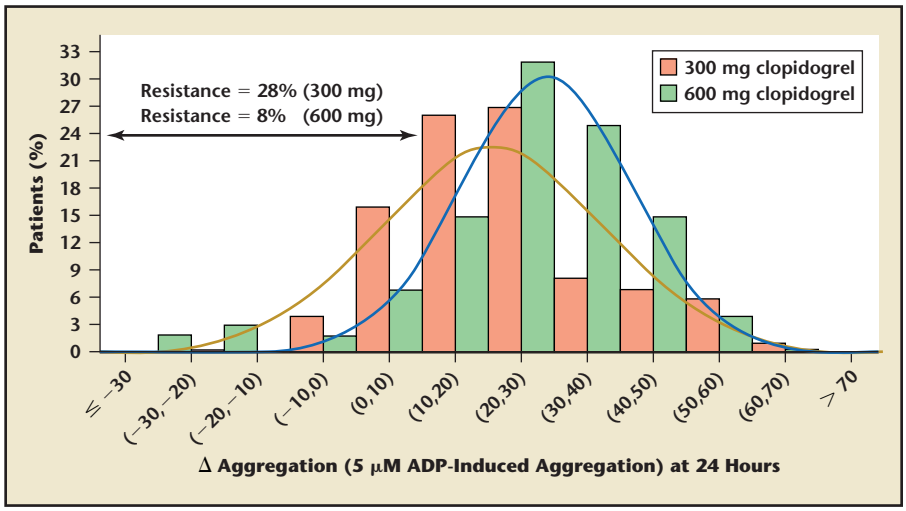


Figure 6. *Distribution of the absolute change in 5 μM adenosine diphosphate (ADP)-induced aggregation (Δ aggregation) and incidence of clopidogrel resistance in patients treated with a 300-mg and 600-mg clopidogrel loading dose. All of the patients under the double-headed arrow meet the definition of clopidogrel resistance. The distribution is shifted rightward and is narrower in the 600-mg group, indicating greater inhibition (responsiveness to clopidogrel) and lower incidence of resistance. Adapted with permission from Gurbel PA et al.*[85] www.medreviews.com

standard doses of clopidogrel. This hypothesis will be formally tested when prasugrel (a new thienopyridine with greater potency than clopidogrel) is compared to clopidogrel in the TRial to assess Improvement in Therapeutic Outcomes by optimizing platelet InhibitioN with prasugrel-Thrombolysis In Myocardial Infarction (TRITON-TIMI) 38.[95] From a safety and convenience standpoint, it would be ideal for an agent to have rapid offset in the setting of bleeding or need for elective surgery, but without the activation of platelets that has been seen with oral GPIIb/IIIa receptor blockers.[96]

Conclusion

Platelet activation and aggregation play important roles in the genesis of cardiovascular events. Inhibition of platelets with aspirin and thienopyridines had resulted in clinical improvements for patients with coronary disease, however, important limitations of each therapy remain.

High incidences of aspirin resistance have been reported using COX-1–specific and COX-1–nonspecific tests, as discussed. In addition, clopidogrel nonresponsiveness has been a consistently observed phenomenon in studies conducted at multiple sites utilizing various $P2Y_{12}$ receptor–specific assays. Noncompliance may also be an important factor related to aspirin and clopidogrel resistance estimates. Emerging data from small studies suggest that patients with high ex vivo platelet reactivity to ADP during and after PCI may be at greatest risk for subsequent ischemic events.

However, at this time there are no uniformly established methods to quantify ex vivo platelet reactivity after clopidogrel and aspirin treatment or to measure the extent of platelet inhibition by clopidogrel and aspirin. Therefore, no specific treatment recommendations are established for patients who exhibit high platelet reactivity during clopidogrel or aspirin therapy or who have poor platelet inhibition by clopidogrel or aspirin. Measurements of platelet function in treated patients with cardiovascular disease may become the standard of care, just as low-density lipoprotein is routinely measured during statin use. The clinical utility of platelet function assays will be determined by definitive, large-scale investigations addressing the link between insufficient platelet inhibition and cardiovascular thrombotic risk. Personalized antithrombotic treatment strategies may follow ex vivo measurements that identify critical pathways influencing thrombotic risk in the individual patient. Currently, treatment of patients who exhibit heightened reactivity of these pathways with specific inhibitors is being investigated in small pilot trials. The bleeding issue associated with prolonged dual antiplatelet therapy is another concern and limitation of existing antiplatelet therapy. At this time, it is uncertain whether there is a threshold of platelet reactivity beyond which ischemic events increase. Similarly, the threshold platelet reactivity for bleeding is unknown. In the near future, new, more potent $P2Y_{12}$ receptor blockers will likely overcome the limitations of clopidogrel. These agents are currently being studied in clinical trials and may represent important advances in the treatment of cardiovascular disease. ■

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Main Points

- Dual antiplatelet therapy with a thienopyridine and aspirin has become the standard of care for patients undergoing percutaneous coronary interventions with stenting, regardless of indication.
- Heightened platelet reactivity despite dual antiplatelet therapy with aspirin and clopidogrel has been associated with stent thrombosis, restenosis, inflammation, myocardial infarction, and other ischemic events following stenting.
- Reports of high incidences of aspirin resistance may have been influenced by methods that do not isolate the response of platelet cyclooxygenase-1 to aspirin.
- Clopidogrel nonresponsiveness has been a consistently observed phenomenon in studies conducted at multiple sites utilizing various $P2Y_{12}$ receptor–specific assays.

therapy in unplanned and elective coronary stenting. The full anticoagulation versus aspirin and ticlopidine (fantastic) study. *Circulation*. 1998;98:1597-1603.
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