REVIEW
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Aspirin and Clopidogrel Resistance in Drug Eluting Stent Era

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ABSTRACT

Platelets play a central role in the pathogenesis of atherothrombosis. Dual antiplatelet therapy with clopidogrel plus aspirin has been shown to reduce ischemic events in patients undergoing percutaneous coronary intervention (PCI) and stenting. Although dual antiplatelet therapy reduces the risk of cardiovascular episodes after PCIs, a substantial number of incidents continue to occur. Many cardiologists have focused their attention to the relationships between the interindividual variability of platelet inhibition after aspirin or clopidogrel administration and major cardiac adverse events such as stent thrombosis. Recent evidence has suggested that "aspirin or clopidogrel resistance" is associated with poor health outcomes (recurrent atherothrombotic events and stent thrombosis) after drug eluting stent (DES) implantation. However, the current clinical guidelines do not support routine screenings for antiplatelet resistance because standardized objective screening has not yet been established. Thus, this review describes the antiplatelet therapy used in PCI and it outlines the mechanism, laboratory tests, clinical impact and treatment options for aspirin and clopidogrel resistance in the DES era. **(Korean Circulation J 2007;37:135-147)**

KEY WORDS : Platelets ; Aspirin ; Clopidogrel ; Drug resistance ; Stents ; Angioplasty.

Introduction

Platelets play a central role in the pathogenesis of atherothrombosis.[1] Thus, achieving platelet inhibition is an important part of managing those patients who suffer from an atherothrombotic event.[2)3)]

Low-dose aspirin is effective for preventing adverse vascular events in patients suffering with acute coronary syndrome stroke and/or peripheral vascular disease. A meta-analysis of 287 randomized studies demonstrated a 25% to 30% reduction of cardiovascular events with administering aspirin, including myocardial infarction (MI), stroke and death.[4] However, aspirin alone in many instances is not sufficient to prevent ischemic events in the high risk patients because aspirin inhibits only the cyclooxygenase pathway and it has no effect on the adenosine diphosphate P_2Y_{12} receptor.[5] Dual antiplatelet therapy with clopidogrel plus aspirin has been shown to reduce ischemic events in patients with unstable angina and MI, and especially when they are undergoing percutaneous coronary intervention(PCI) and stenting.[6)7)]

Despite its proven benefit, the responses of individual patients show considerable heterogeneity to aspirin and to clopidogrel.[8-11)] The recent data shows that adequate antiplatelet effects are not achieved in 5% to 45% of the patients taking aspirin and in 4% to 30% of patients taking clopidogrel,[8-11)] and this suggests that many patients are resistant or partially responsive in the drugs' antiplatelet effect. Thus, measuring the patients' responsiveness to aspirin and clopidogrel may be an important factor in monitoring these drugs' therapeutic efficacy and so improve the cardiovascular outcomes. However, there are no clinical guidelines to deal with treatment failure for the stent thrombosis and recurrent atherothrombotic events that are due to aspirin and clopidogrel resistance.

This review 1) describes the process of platelet activation and antiplatelet therapy for PCI and 2) outlines the mechanism, laboratory tests, clinical impact and treatment options for patients who display aspirin and clopidogrel resistance.

Platelet activation and antiplatelet therapy in PCI

Platelet activation occurs in the patients who undergo PCI as a result of the PCI procedure itself and the underlying atherothrombotic disease. PCI techniques cause denudation of the arterial endothelium and mechanical disruption of the coronary plaque. Following exposure of the subendothium to circulating blood, there is extensive platelet adhesion, platelet activation and platelet aggregation(Fig. 1).[12)13)] Vascular injury and the accom-

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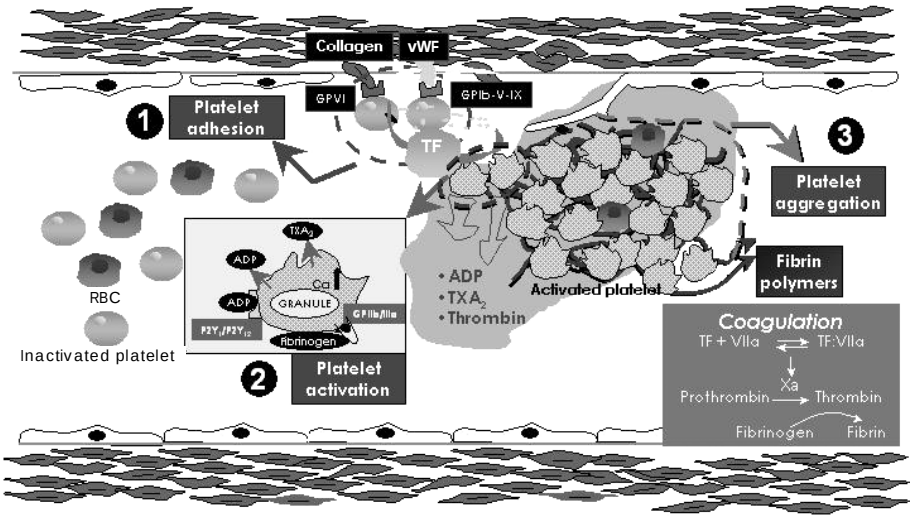

Fig. 1. Platelet adhesion, activation and aggregation. ①: platelet adhesion is mediated by adhesive proteins such as von Willebrand factor (vWF). These adhesive proteins interact with platelet receptors such as glycoprotein Ib complex and glycoprotein VI, which also regulate platelet-leukocyte adhesion. ②: platelets can be activated by adhesion to the arterial wall and by interacting with such circulatory agents as epinephrine, thrombin, serotonin, thromboxane A2 (TXA_2) and adenosine diphosphate (ADP) via specific platelet surface receptors. Platelet activation leads to change of the platelet shape, from a smooth discoid contour into a speculated form. Following the shape change, platelet activation involves secretion of alpha and dense granules (ADP release) within the platelet. Platelet activation also induces phospholipase A2 activation that triggers arachidonic acid metabolism. Platelet cyclooxygenase 1 (COX-1) catalyzes the conversion of arachidonic acid to TXA_2, which enhances platelet activation and vasoconstriction. ③: platelet activation leads to a conformational change in the glycoprotein IIb/IIIa receptor, converting the receptor into a form that can bind fibrinogen and link with other platelets (platelet aggregation). GP: glycoprotein, RBC: red blood cell, TF: tissue factor (Adapted and modified from reference 1).

panying platelet adhesion and aggregation process following PCI might be closely associated with major adverse cardiac events after procedures such as abrupt vessel closure, subacute stent thrombosis and restenosis.[14-17]

Drug-eluting stent(DES) has recently been widely used as a new and effective means to prevent restenosis in daily practice.[18-20] Owing to the possibility of delayed endothelialization and enhanced platelet aggregation after DES implantation, some reports have warned about the possibility of a higher risk of stent thrombosis.[21] The major pathologic finding of DES, compared to that of bare metal stents, was evidence of significant delay of arterial healing, as manifested by persistent fibrin deposition and partial endothelialization. This give rise to a potent thrombogenic stimulus and pathologic substrate underlying the phenomenon of stent thrombosis.[21] This prothrombotic environment is maintained for months after the initial ischemic event and it's likely to contribute to the recurrence of ischemic events, as determined on long-term follow-up.

Preventing recurrent ischemic events after PCI requires antiplatelet therapy as an integral component of patient management during and after PCI.[22] Aspirin irreversibly inhibits COX-1 by acetylating a serine residue at position 530, thereby preventing the conversion of arachidonate to the unstable prostaglandin(PG) intermediate PGH_2, and this PG intermediate PGH_2 is then converted to TXA_2, a potent vasoconstrictor and platelet agonist(Fig. 2).[23] Aspirin exerts its inhibitory effect within 60 minutes of oral administration, and its effect on platelet inhibition lasts for seven days after the last dosage.[23] Aspirin pretreatment is effective in reducing perioperative ischemic events during balloon angioplasty.[24] The minimum effective aspirin dosage in the setting of PCI has not been established. Because the GI side effects of aspirin are dose related, an empiric dose of aspirin (75 to 325 mg) is administered at least two hour prior to the procedure. ACC/AHA/SCAI 2005 updated guidelines for the recommended use of aspirin for PCI are shown below.[22]

Class I

1) Those patients already taking daily chronic aspirin therapy should take 75 to 325 mg of aspirin before undergoing a PCI procedure(Level of Evidence: A).

2) 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).

3) After the PCI procedure, those patients with neither aspirin resistance, allergy or an increased risk of bleeding should be given aspirin 325 mg daily for at least 1 month after baremetal stent implantation, for 3 months after sirolimus-eluting stent implantation and for 6 months after paclitaxel-eluting stent implantation. After this, daily long-term aspirin use should be continued indefinitely at a dose of 75 to 162 mg(Level of Evidence: B).

Despite of its proven benefits, aspirin is a relatively weak antiplatelet agent and inhibits only one of several pathways that lead to platelet activation, that is, genera-

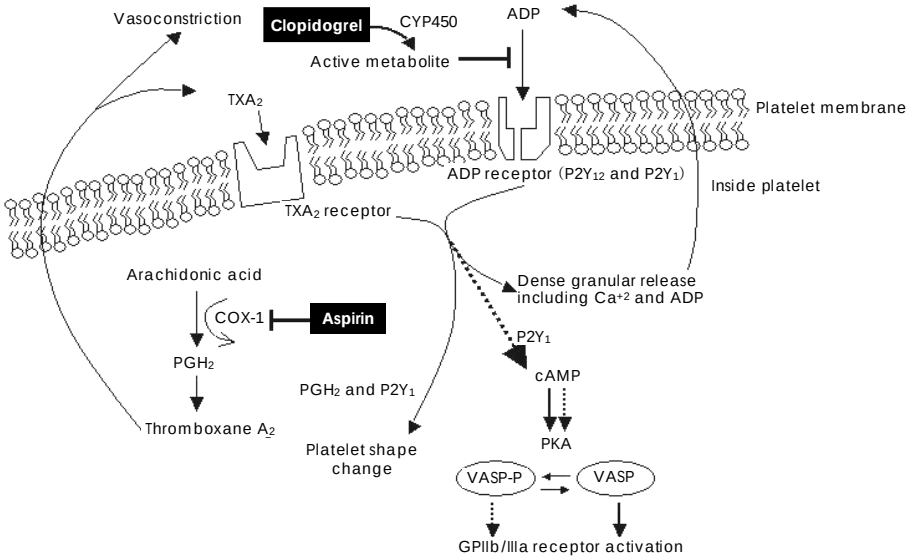

Fig. 2. Schematic illustration of the pharmacologic sites of aspirin and clopidogrel. COX-1: cycloxygenase-1, PGH_2: prostaglandin H_2, TXA_2: thromboxane A_2, ADP: adenosine diphosphate, PKA: protein kinase-A, VASP: vasodilator stimulated phosphoprotein, cAMP: cyclic adenosine monophosphate, CYP450: cytochrome P450 (Adapted from reference 41).

tion of TXA_2. The thienopyridines such as clopidogrel and ticlopidine irreversibly inactivate the platelet ADP receptor $P2Y_{12}$, which is one of the two G-protein coupled receptors($P2Y_{12}$ & $P2Y_1$) that are expressed on the platelet membrane(Fig. 2), and the combined action of these is necessary for platelet activation by ADP. $P2Y_{12}$ is negatively coupled to adenylyl cyclase through Gi, and this decreases the level of cyclic adenosine monophosphate(cAMP) in platelets. The decrease in cAMP production and the downstream pathway inhibit platelet activation.[25-29] Because of the different mechanisms of thienopyridines, as compared to those of aspirin, the thienopyridines acts additively or synergistically with aspirin through an independent mechanism of platelet inactivation.

Ticlopidine was the first of this new class of antiplatelet agents to be discovered. The CATS[30] and TASS[31] trials have demonstrated the effectiveness of ticlopidine for reducing the risk of thrombotic events in patients with atherothrombotic disease. In addition, the combination of aspirin and ticlopidine reduces the risk of subacute thrombosis and this reduced risk is associated with a lower risk of bleeding, as compared with an anticoagulant regimen(aspirin with warfarin) after stent implantation.[32][33] However, ticlopidine has been associated with a rare but severe incidence of neutopenia(about 1%) and bone marrow aplasia, and this has curbed its routine use in PCI.[30][34][35]

Clopidogrel is more widely used than ticlopidine in current clinical practice because it offers better safety, tolerability and a faster onset of action with similar efficacy for treating stent thrombosis and periprocedural ischemia.[36][37] Theoretically, adding clopidogrel to aspirin can additively enhance the inhibition of platelet activation, along with decreased adverse drug reactions, via inhibition of the ADP-induced platelet activation in a background of TXA_2 inhibition. Indeed, the CURE[38] trial introduced for the first time the benefit of adding clopidogrel to aspirin(a 20% reduction of the relative risk of MI, stroke and death) compared to using aspirin only for patients with acute coronary syndrome. In the CURE trial, there were not significantly more patients who experienced episodes of life-threatening bleeding (2.2% vs. 1.8%; p=0.13) or hemorrhagic strokes(0.1% vs. 0.1%).

Trials such as the PCI CURE,[39] CREDO[7] and PCI CLARITY[40] verified the concept of employing aspirin and clopidogrel as dual antiplatelet therapy for secondary prevention after elective or urgent PCI in patients with unstable angina, non-ST elevation and ST elevation MI. Clopidogrel combined with aspirin is currently the drug regimen of choice to prevent thrombosis after coronary stent implantation. The ACC/AHA/SCAI 2005 updated guideline for PCI recommended using clopidogrel as written below.[22]

Class I

1) 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).

2) For the patients who have undergone PCI, clopidogrel 75 mg daily should be given for at least 1 month after bare-metal stent implantation, 3 months after sirolimus stent implantatio and 6 months after paclitaxel

stent implantation, and ideally for up to 12 months in patients who are not at a high risk of bleeding(Level of Evidence: B).

Class IIa

1) If clopidogrel is given at the time of the procedure, supplementation with GP IIb/IIIa receptor antagonists can be beneficial to facilitate earlier platelet inhibition than is achieved with administering clopidogrel alone (Level of Evidence: B).

2) When a loading dose of clopidogrel is administered, a regimen of greater than 300 mg is reasonable to more rapidly achieving higher levels of antiplatelet activity, but the efficacy and safety compared with a 300-mg loading dose are less established(Level of Evidence: C).

Class IIb

For the patients whom subacute thrombosis may be catastrophic or lethal(unprotected left main, bifurcating left main or the last patent coronary vessel), performing platelet aggregation studies may be considered and the dose of clopidogrel may be increased to 150 mg per day if less than 50% inhibition of platelet aggregation is demonstrated(Level of Evidence: C).

Aspirin and clopidogrel resistance

Despite aspirin's and clopidogrel's proven benefit for reducing atherothrombotic complications, recurrent CV events still occur in the patients taking these drugs. A lot of these patients are resistant or only partially responsive to aspirin or clopidogrel. The term "resistance" to a drug should be used when a drug is unable to hit its pharmacological target. Thus, the term aspirin or clopidogrel resistance has been used to describe the inability of aspirin or clopidogrel to inhibit platelet aggregation, as is demonstrated by platelet function tests(biochemical aspirin or clopidogrel resistance). The term has also been used to describe the failure of these drugs to prevent of cardiovascular events despite taking aspirin or clopidogrel (clinical aspirin or clopidogrel resistance). No consensus exists on whether the definition is based on laboratory evidence(e.g., biochemical resistance), clinical outcomes (e.g., clinical treatment failure) or both.[41]

The possible mechanisms of aspirin and clopidogrel resistance include alterations in genetic, pharmacokinetics and platelet properties(Tables 1 and 4). Evidence from small clinical studies suggests that a decreased response or resistance to antiplatelet drugs is associated with subsequent CV events.[8][42-48] However, the small sample size, the differences in patient characteristics and the use of different assays to quantify the antiplatelet effects make it difficult to draw any definitive conclusions between low responsiveness to aspirin or clopidogrel and thrombotic events. Thus, large scale randomized studies of antiplatelet resistance in patients suffering with atherothrombosis are needed.

Table 1. Possible mechanisms of aspirin resistance

1. Inadequate aspirin dosage
2. Non-absorption
3. Noncompliance with therapy
4. Formulations of aspirin with low bioavailability (enteric-coated aspirin)
5. Drug-drug interactions with some NSAIDs (e.g., ibuprofen)
6. Cigarette smoking
7. Diabetes mellitus
8. Hypercholesterolemia
9. Catecholamine surge (exercise or stress)
10. Formation of isoprostanes
11. Increased platelet sensitivity to adenosine diphosphate and collagen
12. Polymorphisms in the glycoprotein IIb/IIIa receptor gene and the COX-1 gene, the collagen receptor gene and the vWF gene
13. Inadequate blockade of erythrocyte and monocyte/macrophage activation
14. Decreased platelet sensitivity to aspirin over time (tolerance)

NSAID: including noncompliance, hyperglycemia, hypercholesterolemia, smoking and drug interaction, COX-1: cyclooxygenase 1, vWF: von Willebrand factor (Adapted from reference)

Aspirin resistance

The mechanisms of aspirin resistance are multifactorial(Table 1) because multiple factors affect platelet aggregation with using aspirin, including poor bioavailability(non-compliance, underdosing, poor absorption and drug interaction etc.), accelerated platelet turnover, genetic factors and other factors(exercise, serum cholesterol, cigarette smoking and patient posture etc.). The common clinical cause of aspirin resistance, as well as clopidogrel resistance, in patients with coronary artery stents is premature discontinuation of antiplatelet therapy (non-compliance).[49] This markedly increases the risk of stent thrombosis, which is a catastrophic event that frequently leads to MI and death. The factors contributing to premature cessation of antiplatelet therapy include the cost of the drugs, the physician/dentist instructions to patents to discontinue therapy before procedures and inadequate patient education and understanding about the importance of continuing therapy.[49]

Moreover, drug-to-drug interactions may reduce the efficacy of aspirin.[50] Ibuprofen, for example, can adhere to the COX-1 binding site of aspirin, and so it may block aspirin's access to the active site of COX-1. Antiplatelet activities are significantly deceased with the concomitant administration of ibuprofen and aspirin. However, another NSAIDs(diclofenac and rofecoxib) showed no or minimal effect on inhibition of platelet aggregation when they were administered with aspirin.[50][51] Another possible mechanism for aspirin resistance is the multiple isoforms of COX-2. Mature platelets contain only the COX-1 isoform, but newly formed platelets

Table 2. Prospective studies regarding aspirin resistance

Population	Method	ASA dose	Main findings
488 cases treated with aspirin who had vascular disease during 5 years of follow-up[8]	Urinary 11-dehydro thromboxane B_2 levels	75-325 mg	The upper quartile had a 2-times-higher risk of MI (OR: 2.0, 95% CI: 1.2 to 3.4, p=0.006) and a 3.5-times-higher risk of CV death (OR: 3.5, 95% CI: 1.7 to 7.4, p<0.001) than those in the lower quartile
2-year follow-up study with a cohort of 326 stable CV patients[44]	Optical platelet aggregometry	325 mg/day	Aspirin resistance was associated with a 4.1-fold excess adjusted hazard for serious vascular events (HR: 4.1, 95% CI: 1.4-12.1)
2-year follow-up study with 202 post-MI patients[60]	PFA-100	160 mg/day vs. aspirin 75 mg and warfarin	A tendency for higher event rates in non-responders as compared to responders (36% vs. 24%, p=0.28)
151 patients with CAD who presented for non-urgent PCI[42]	VerifyNow Aspirin Assay ARU >550	75-325 mg	Aspirin resistance (OR: 2.9, p=0.015) was the independent predictor of CK-MB elevation after PCI

ASA: aspirin, CV: cardiovascular, CAD: coronary artery disease, MI: myocardial infarction, PCI: percutaneous coronary intervention, PFA: platelet function analyzer, ARU: aspirin reaction unit, OR: odds ratio, CI: confidence interval, HR: hazard ratio

(10% of the circulating platelets) contain the COX-2 isoform.[52] During periods of high platelet turnover, COX-2-derived TXA may play an unrecognized role in inflammatory and hemostatic responses. Under this condition, aspirin may inadequately inhibit COX-2-derived platelet activation.[53]

Genetics also play a role in patients' responses to aspirin as polymorphisms of the platelet membrane glycoproteins, such as the PL(A1/A2) gene that encodes glycoprotein IIIa, have been associated with an attenuated response to aspirin.[54][55] Polymorphisms of the von Willebrand Factor(vWF) gene, the P_2Y_{12} gene and the COX-1 gene can affect the response to aspirin.[55][56] Despite these findings, the impact of polymorphisms on the response to aspirin remain controversial. Other proposed clinical conditions of aspirin resistance include acute coronary syndrome, congestive heart failure, hyperglycemia(by increasing oxidative stress), hypercholesterolemia and catecholamine surge(exercise or stress) can also affect platelets' responsiveness to asprin.[57-59]

Clinical data of aspirin resistance in cardiovascular disease

Several recent prospective studies have shown an association between biochemical aspirin resistance and the CV disease(Table 2).

In a subgroup study of aspirin-treated patients from the HOPE trial,[8] investigators measured the urinary 11-dehydro thromboxane B_2 levels, which are a marker of in vivo thromboxane generation, in 488 patients who were treated with aspirin and who suffered MI, stroke or CV death during 5 years of follow-up. Those patients in the upper quartile had a 2-times higher risk of MI(OR: 2.0, 95% CI: 1.2 to 3.4) and a 3.5-times higher risk of CV death(OR: 3.5, 95% CI: 1.7 to 7.4) than those patients in the lower quartile. These findings suggested that the laboratory evidence of aspirin resistance(that is, the 11-dehydro thromboxane B_2 level) was independent of the conventional risk factors for atherothrombotic vascular disease.

Gum et al.[44] performed a 2-year follow-up study with a cohort of 326 stable cardiovascular patients who received aspirin 325 mg/day. Baseline aspirin resistance was determined by optical platelet aggregometry, which is the "gold standard" for assessing the response to aspirin. Aspirin resistance was defined as a mean aggregation of>70% with 10 microM ADP and>20% with 0.5 mg/mL arachidonic acid. Multivariate analysis showed that aspirin resistance was associated with a 4.1-fold excess adjusted hazard of serious vascular events (OR: 4.1, 95% CI: 1.4-12.1).

Anderson et al.[60] investigated the prognostic values of aspirin resistance as evaluated by the platelet function anualyzer 100 level in 202 post-MI patients with 4 years follow up. There was a tendency for higher event rates in non-responders as compared to the responders(36% vs. 24%, respectively, p=0.28).

Chen et al.[42] explored the effect of aspirin resistance on the outcomes of patients undergoing PCI. Using the VerifyNow Aspirin Assay, they categorized 151 patients with CAD and who underwent non-emergency PCI. The incidence of any CK-MB elevation was 51.7% in the aspirin-resistant patients and 24.6% in the aspirin-sensitive patients(p=0.006). Multivariate analysis revealed aspirin resistance(OR: 2.9, 95% CI: 1.2 to 6.9, p=0.015) to be independent predictors of CK-MB elevation after PCI. This study shows that patients with aspirin resistance, as measured by a point-of-care assay, have an increased risk of myonecrosis following non-urgent PCI despite being adequately pretreated with clopidogrel.

Diagnosis of aspirin resistance

The techniques for measuring aspirin resistance include the bleeding time,[61] optical platelet aggregometry,[9] measurement of the platelet aggregation ratios,[62] the platelet reactivity index,[63] the TXA_2 metabolite level,[8] flow cytometry,[64] the Platelet Function Analyzer 100 (PFA-100; Dade Behring Inc., DE)[9][44][60] and the VerifyNow® assay(Accumetrics, CA).[65][66] The vast majority

of studies reporting the occurrence of aspirin resistance in different clinical settings have relied on ex vivo measurement of platelet function with using one or more of the following techniques, light transmittance aggregometry(LTA), the PFA-100 and (3) the VerifyNow® Aspirin Assay(Table 3). Each of these techniques challenges the capacity of blood platelets to respond to an aggregating stimulus such as arachidonic acid, which is added at various concentrations in a largely artificial environment.

In case of the VerifyNow® Aspirin Assay, arachidonic acid is used as the agonist. This assay is aspirin specific because arachidonic acid-induced platelet aggregation requires the activity of cyclooxygenase-1, which is specifically blocked by aspirin. The VerifyNow® Aspirin Assay has shown excellent correlation with optical aggregometry with using whole blood, but investigation is needed on the clinical impact of resistance(defined by the VerifyNow® Assay as an aspirin resistance unit(ARU) >550) and the rate of subsequent cardiovascular events. Advantages of the VerifyNow® system include point-of-care use, simplicity, rapidity(results in 5 minutes), low sample volume, and no sample preparation.[65-67]

Table 3. Laboratory assays for aspirin and clopidogrel resistance

Aspirin resistance
In vivo (metabolite of thromboxane)
Serum thromboxane B_2
Urinary 11-dehyro thromboxane B_2
Ex vivo (arachidonic acid stimulus)
VerifyNow® Aspirin assay
Light transmittance aggregometry
Platelet surface P-selectin, GP IIb-IIIa, leukocyte-platelet aggregates
Platelet work
Ex vivo (others)
Platelet function analyzer 100 (PFA-100)
Clopidogrel resistance
In vivo (P_2Y_{12} signal dependant metaboite)
Vasodilator stimulated phosphoprotein (VASP)
Ex vivo (ADP stimulus)
VerifyNow® P_2Y_{12} assay
Light transmittance aggregometry
Platelet surface P-selectin, GP IIb-IIIa, leukocyte-platelet aggregates
Platelet work

GP: glycoprotein, ADP: adenosine diphosphate (Adapted from reference)

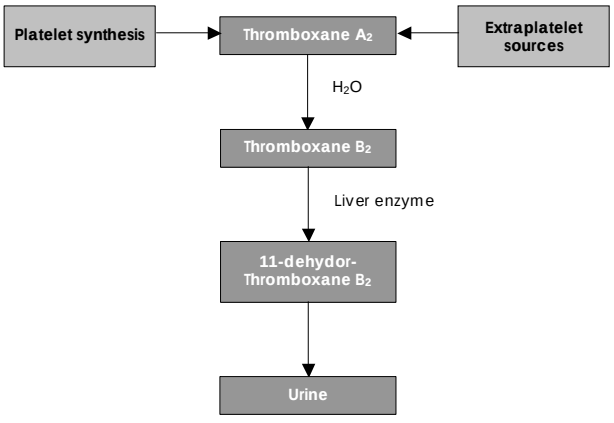

Fig. 3. Metabolic fate of thromboxane A_2 in vivo.

Different methods for testing platelet function have reported a wide range of aspirin resistance(5-60%) in selected populations, with poor concordance among those methods. Moreover, whether changes in these functional indexes accurately reflect platelet activation and inhibition in vivo is currently unclear. Measurements of serum thromoboxane B_2 and urinary 11-dehydro-thromboxane B_2 provide reliable information on the time-integrated index of TXA_2 biosynthesis in vivo(Fig. 3).[68] These measurements have been extensively used to characterize the clinical pharmacology of aspirin as an antiplatelet drug.[68][69] One limitation of these measurements was the inability to differentiate between resistance or low response to the aspirin regimen and the aspirin-insensitive sources of TXA_2 biosynthesis(COX2-induced TXA).

Treatment of aspirin resistance

The treatment of aspirin resistance is as yet undefined. An initial approach of aspirin resistance is to determine any correctable cause of resistance, including noncompliance, hyperglycemia, hypercholesterolemia, smoking and drug interaction(NSAIDs). It remains unproven that an increased dosage of aspirin may overcome aspirin resistance in an individual patient. A study on sixty patients who were taking aspirin 81 mg/day showed a low platelet response of 8%, but when the dose was increased 325 mg/day, then the platelet response was improved.[70] This data suggests that increasing the dose of aspirin would be a way to ensure an adequate platelet response(Fig. 4).

The change or addition of clopidogrel might be logical because of its distinct mechanism of action. The Clopidogrel vs. Aspirin in Patients at Risk of Ischemic Events(CAPRIE) study revealed the modest superiority of clopidogrel(31.2% relative risk reduction) compare to aspirin.[71] A substudy of the CHARISMA trial will determine the benefit of clopidogrel for patients with aspirin resistance. Potential alternative drugs or new drugs such as thromboxane receptor antagonists(BM-573)[72] are currently under investigation.

Clopidogrel resistance

Clopidogrel is an inactive prodrug that requires in vivo conversion in the liver by the cytochrome P450 3A4 enzyme system to an active metabolite, and this acts via irreversible antagonism of the platelet P_2Y_{12} ADP receptors.[27][67] Therefore, its pharmacological effect can be detected only some time after its first administration and the plasma levels of the active metabolite vary widely

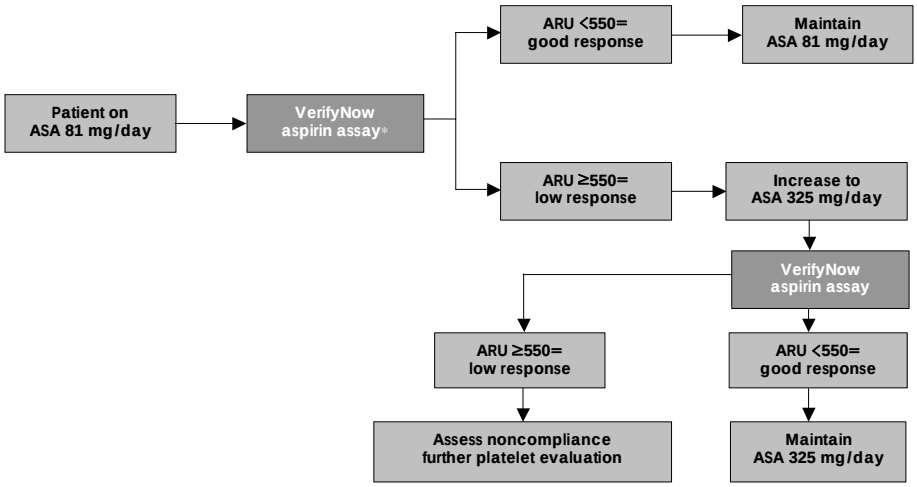

Fig. 4. Critical pathway for aspirin (ASA) response with using a point-of-care system (VerifyNow Aspirin assay). ARU: aspirin reaction unit (Adapted from reference).

Table 4. Possible mechanisms of clopidogrel resistance

1. Inadequate clopidogrel dosage
2. Non-absorption
3. Noncompliance with therapy
4. Drug-drug interactions involving cytochrome P450 3As (e.g., some statins)
5. Acute coronary syndrome (increased baseline platelet activity)
6. Insulin resistance (e.g., diabetes)
7. Increased body mass index
8. Polymorphisms in the cytochrome P450 3A and P_2Y_{12} genes

among subjects due to individual variability of absorption and metabolism. Lau et al.[73][74] showed that the interindividual variability of platelet inhibition by clopidogrel correlated well with the metabolic activity of the hepatic cytochrome P450 enzyme($r=-0.6$, $p=0.003$), and this might be a major component of the variable response to clopidogrel. Some statins such as atorvastatin have the ability to inhibit the cytochrome P450 3A4 enzyme system, which can result in possible interaction if administered concomitantly with clopidogrel.[50][73] However, the Interaction of Atorvastatin and Clopidogrel study[75] did not show any interaction between atorvastatin and clopidogrel. Recent evidence demonstrated that there was no difference in clinical outcomes between the patients taking clopidogrel and statins that were metabolized by or not metabolized by the cytochrome P450 3A4 enzyme system.[76] The conflicting ex vivo and clinical data regarding clopidogrel and some statins is still controversial.

Other possible mechanisms for clopidogrel resistance have been proposed(Table 4), including poor bioavailability(non-compliance, underdosing, poor absorption, drug interaction etc.), accelerated platelet turnover, genetic factors(P_2Y_{12} H_2 haplotype[77]) and clinical factors(acute coronary syndrome, increased body mass index, insulin resistance[78][79] etc.).

Clinical data of clopidogrel resistance in PCI

Several studies have attempted to characterize the relationship between response variability and clinical outcomes(Table 5). Matetzky et al.[46] prospectively studied 60 consecutive patients who underwent primary PCI with stenting for treating acute ST-segment-elevation MI to determine whether the variability in response to clopidogrel affects the clinical outcomes. They showed that up to 25% under-going primary PCI with stenting are resistant to clopidogrel and so they may be at an increased risk for recurrent CV events. Gurbel et al.[10] measured the platelet aggregation(5 and 20 μM/L ADP), the activation of GP IIb/IIIa(PAC-1 antibody), and the expression of p-selectin in patients undergoing elective coronary stenting($n=96$) at baseline and at 2 hours, 24 hours, 5 days and 30 days after stenting. Clopidogrel resistance was present in 31% and 15% of patients at 5 and 30 days, respectively. They suggested that inter-individual variability in the platelet inhibitory response to clopidogrel occurs in patients undergoing elective coronary stenting.

Clopidogrel effect on platelet reactivity in patients with stent thrombosis(CREST) study[45] investigated whether patients who suffered subacute stent thrombosis(SAT) have higher post-treatment reactivity than those patients who do not suffer from stent thrombosis. The LTA induced by ADP and arachidonic acid, the total and activated GP IIb/IIIa levels after stimulation with ADP, and VASP phosphorylation levels to measure the P_2Y_{12} receptor inhibition were determined($n=20$) and compared with an agematched group of patients without SAT($n=100$). The SAT patients had higher mean platelet reactivity than those patients without SAT according to all measurements($p<0.05$); $49\pm4\%$ versus $33\pm2\%$, respectively, for the 5 μM/L ADP-induced aggregation and $65\pm3\%$ versus $51\pm2\%$, respectively, for the 20 μM/L ADP-induced aggregation($p<0.001$), $69\pm5\%$ versus $46\pm9\%$, respectively, for the P_2Y_{12} reactivity ratio($p=$

Table 5. Prospective studies regarding clopidogrel resistance

Population	Method	Clopidogrel dose	Main findings
60 patients who underwent primary PCI with stenting with 6-month follow-up[46]	ADP-induced aggregation & Cone-and-plate (let) analyzer method	75 mg	40% of the patients in the first quartile of resistance sustained a recurrent CV event, and only 1 patient in the second quartile and none in the third and f ourth quartiles suffered a CV event
96 patients who underwent elective coronary stenting (n=96)[10]	ADP-induced aggregation, the activation of GP IIb/IIIa and p-selectin	300 mg (loading dose), 75 mg (maintenance dose)	Clopidogrel resistance was present in 31% and 15% of the patients at 5 and 30 days, respectively.
20 patients who suffered subacute stent thrombosis (SAT) [45]	ADP-induced aggregation, GP IIb/IIIa after stimulation with ADP,and the VASP levels	75 mg	The SAT patients had higher mean platelet reactivity than those patients without SAT by all measurements ($p<0.05$): 49±4% versus 33±2%, respectively, for 5 μM ADP-induced aggregation and 138±19 mean fluorescence intensity (MFI) versus 42±4 MFI for the stimulated GP IIb/IIIa expression ($p<0.001$)
106 non-ST segment elevation ACS patients undergoing PCI with stenting[80]	ADP-induced aggregation, and the highest quartile (quartile 4) were defined as the 'low-responders'	75 mg	The clinical outcome was significantly associated with the platelet response to clopidogrel [quartile 4 vs. quartiles 1, 2 and 3: odds ratio and (95% CI): 22.4 and (4.6-109)].

PCI: percutaneous coronary intervention, ADP: adenosine diphosphate, VASP: vasodilator stimulated phosphoprotein, CI: confidence interval, ACS: acute coronary syndrome, GP: glycoprotein, CV: cardiovascular, SAT: subacute stent thrombosis

0.03), and 138±19 mean fluorescence intensity(MFI) versus 42±4 MFI, respectively, for the stimulated GP IIb/IIIa expression($p<0.001$). They suggested that high post-treatment platelet reactivity and incomplete inhibition are risk factors for SAT.

Cuisset et al.[80] prospectively studied the platelet response to both clopidogrel and aspirin in 106 non-ST segment elevation acute coronary syndrome patients who underwent PCI with stenting. A single post-treatment blood sample was obtained just before PCI and this was analyzed by platelet aggregometry with using both ADP and arachidonic acid. Patients of the highest quartile (quartile 4) were defined as the 'low-responders'. The clinical outcome was significantly associated with the platelet response to clopidogrel [Quartile 4 vs. the 1, 2 and 3 quartiles; odds ratio and(95% CI): 22.4 and(4.6-109)]. They concluded that a post-treatment ADP-induced platelet aggregation test performed just before PCI identifies the low responders to dual antiplatelet therapy, and these low responders have an increased risk of recurrent CV events. Despite accumulating evidence showing the relationship between clopidogrel resistance and clinical outcomes, the major limitations of these studies are 1) the number of study subjects and MACEs are relatively low in these studies, and 2) the definition of clopidogrel resistance varied depending on the assay methods.

Another issue is the loading dose effect on clopidogrel responses during early invasive procedures for treating acute coronary syndrome. Gurbel et al.[81] investigated the responsiveness to clopidogrel and the post-treatment platelet aggregation(post-PA) in patients undergoing stenting(n=190) and who were randomly treated with either a 300 mg or a 600 mg clopidogrel loading dose. Non-responsiveness was lower after 600 mg compared to the 300 mg dose(8% vs. 28% and 8% vs. 32% with 5 and 20 μM ADP, respectively, $p<0.001$). They concluded that a 600 mg clopidogrel loading dose reduces the incidence of non responsiveness and high post-PA as compared to a 300 mg dose. The ISAR-CHOICE study[82] has compared 300, 600 and 900 mg loading doses of clopidogrel. Loading with 600 mg reduces the incidence of nonresponsiveness. However, with administration of 900 mg clopidogrel, no further increase in plasma concentrations of active metabolite and clopidogrel($p=0.38$) was achieved and no further suppression of adenosine diphosphate-induced(5 and 20 μM/L) platelet aggregation 4 hours after drug administration was achieved when compared with the administration of 600 mg clopidogrel ($p=0.59$ and 0.39). They showed that single doses of clopidogrel higher than 600 mg are not associated with any additional significant suppression of platelet function because of the limited absorption of clopidogrel.

The clinical utility of a 600 mg clopidogrel loading dose has been confirmed in the ARMYDA-2 study.[83] A total of 255 patients scheduled to undergo PCI were randomized to a 600 mg(n=126) or 300 mg(n=129) loading regimen of clopidogrel. The primary end point (death, MI and TVR) occurred in 4% of the patients in the high loading dose group versus 12% of those in the conventional loading dose group($p=0.041$) and this difference was entirely due to periprocedural MI. They concluded that pretreatment with a 600 mg loading dose

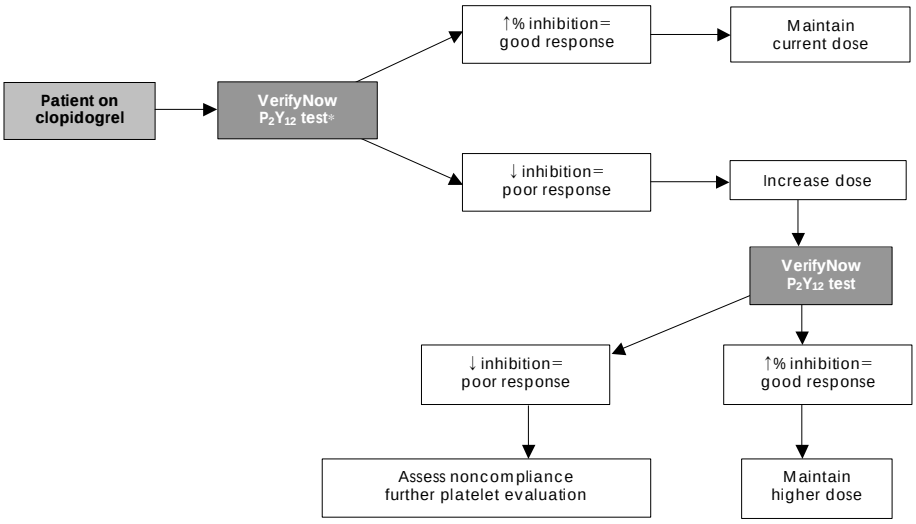

Fig. 5. Critical pathway for clopidogrel response using a point-of-care system (VerifyNow P_2Y_{12} test) (Adapted from reference).

of clopidogrel 4 to 8 hours before the procedure is safe and, as compared with the conventional 300 mg dose, it significantly reduced periprocedural MI in patients undergoing PCI.

On the basis of these studies, a high loading dose (600 mg) of clopidogrel might be beneficial to decrease the clopidogrel resistance and improve the clinical outcomes. The OASIS-7 study(an ongoing trial) will determine the clinical benefits of a high loading dose of clopidogrel for patients with acute coronary syndrome undergoing PCI.

Techniques for measuring clopidogrel resistance

Light transmission aggregometry(LTA) is a "gold standard" for investigations on the variability of the response to clopidogrel. The LTA assay is time-consuming, technically demanding and it is not available in clinical settings. Thus, point-of-care assay systems have been used in clinical settings. These include the VerifyNow device, which is similar to the principle of LTA, the PFA 100 system, which measures time for the occlusion of an aperture by platelet aggregation in response to a specific stimulus, and the Plateletworks assay(Helena Lab. CA), which calculates platelet aggregation on the basis of measurements of platelet counts. A biomarker of clopidogrel response is vasodilator-stimulated phosphoprotein(VASP), which is phospholylated or dephospholylated in the presence of P_2Y_{12} stimulation(Table 2).

Treatment of clopidogrel resistance

The treatment of clopidogrel resistance is as yet undefined. An initial approach to clopidogrel resistance was to correct the causes of resistance, including noncompliance, insulin resistance(metabolic syndrome) and drug interaction(statins). However, the other current practical strategies to overcome clopidogrel resistance would be a double dose of clopidogrel, and the addition of cilostazol or GP IIb/IIIa inhibitor

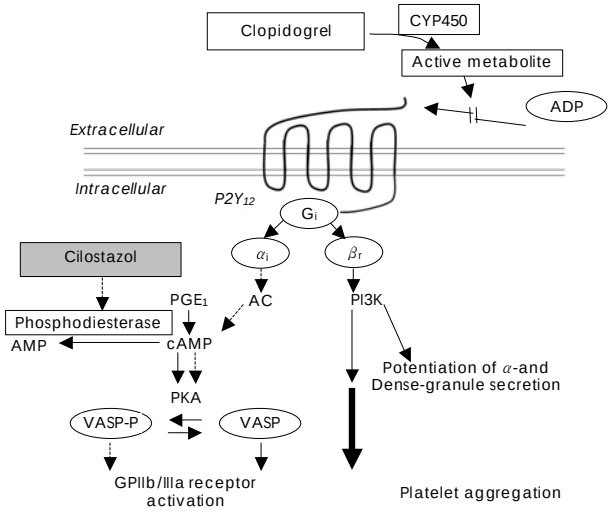

Fig. 6. Proposed mechanism of improving clopidogrel resistance by cilostazol. ADP: adenosine diphosphate, PKA: protein kinase-A, VASP: vasodilator stimulated phosphoprotein, AC: adenylate cyclase, cAMP: cyclic adenosine monophosphate. PGE_1: alprostadil (Adapted from reference).

A recent small study has evaluated the efficiency of a 150 mg oral maintenance dose of clopidogrel.[84] Sixty patients after pre-treatment with 600 mg of clopidogrel and they were within 12 h after successful PCI were included in this trial. These patients were allocated to receive one of two clopidogrel daily maintenance doses (75 or 150 mg) for 30 days in a double-blind randomized manner. The platelet function was evaluated 30 days after the intervention, as was determined with performing optical aggregometry and the VerifyNow P_2Y_{12} assay. The maximal 5 μM ADP-induced platelet aggregation 30 days after PCI in the group treated with 150 mg/day clopidogrel($45.1 \pm 20.9\%$) was significantly lower than that in the group treated with 75 mg/day($65.3 \pm 12.1\%$, $p<0.001$). The VerifyNow P_2Y_{12} assay also indicated a

higher degree of platelet function inhibition for the group treated with 150 mg/day as compared to that in the group treated with 75 mg/day(p=0.004). The authors of that study concluded that administration of a 150 mg oral maintenance dose of clopidogrel results in more intense inhibition of platelet aggregation than administration of the currently recommended 75 mg maintenance dose. This data suggest that testing for clopidogrel response and increasing the dose of clopidogrel would be a way to ensure an adequate platelet response(Fig. 5).

Cilostazol is a potent oral antiplatelet agent with a rapid onset of action, and it selectively inhibits phosphodiesterase III and increases the cAMP levels in the platelet. The increase in the cAMP blocks all the activating pathways in platelets, including the ADP-induced platelet activations(Fig. 6).[85-87] Lee et al.[88] have compared the clinical benefit, for patients undergoing PCI, between dual antiplatelet therapy(aspirin plus clopidogrel or ticlopidine, group I, n=1,597) and triple antiplatelet therapy (aspirin plus clopidogrel or ticlopidine plus cilostazol, group II, n=1,415). Death, myocardial infarction, target lesion revascularization or stent thrombosis within 30 days occurred in 0.8% of the patients in group I and this occurred in 0.3% of the patients in group II(p=0.085). Stent thrombosis within 30 days was significantly lower in group II(n=1, 0.1%) than that in group I(n=9, 0.5%, p=0.024). The independent predictors of stent thrombosis were primary stenting(OR: 7.9, 95% CI: 2.0 to 30.8, p=0.003) and triple therapy(OR: 0.12, 95% CI: 0.015 to 0.98, p=0.048). They concluded that triple antiplatelet therapy seemed to be more effective in preventing thrombotic complications after stenting without an increased risk of side effects.

We recently evaluated the additional effect of cilostazol for the ADP-induced platelet aggregation in 60 patients undergoing primary PCI(unpublished data). These patients were randomly assigned to 2 groups: the dual antiplatelet therapy(aspirin and clopidogrel) group and the triple regimen(dual plus cilostazol) group. The aspirin and clopidogrel resistances were evaluated by VerifyNow tests. The aspirin reaction units (ARU) were similar in both groups(dual: 421.1±49.6 vs. triple: 426.4±62.1, p=0.717). However, the %inhibition of P_2Y_{12} was significantly higher in the triple group than that in the dual group(dual: 24.2±21.7% vs. triple: 40.5(21.0%, p=0.006)). The incidence of low clopidogree response was also higher in the dual group than that in the triple group (79.3% vs. 46.4%, p=0.010). The soluble CD40L level of the triple regimen group was markedly declined in the patients with a low response at 24 hours(186.4±154.4 pg/mL vs. 65.3±15.3 pg/mL, respectively, p=0.009, respectively). Additional administration of cilostazol additionally inhibited the ADP-induced platelet aggregation and it lowered the soluble CD40L level in the early phase of primary PCI. These results suggested that cilostazol can improve the clopidogrel resistance in patients suffering with acute myocardial infarction.

One another logical approach to overcome clopidogrel resistance is using GP IIb/IIIa antagonists because of their distinct mechanism of action. Dalby et al.[89] investigated 32 Non-STEMI patients who were treated with aspirin and enoxaparin by performing flow cytometry to define the parameters of platelet activation, and they used a panel of agonists before clopidogrel, after clopidogrel and during an eptifibatide infusion following clopidogrel loading. After platelet activation with administering ADP, thrombin receptor-activating peptide or U46-619, relative reductions in the conformationally activated GP IIb/IIIa receptor expression(as evaluated with PAC-1) of 48%, 43% and 33%, respectively(all p<0.0001), were seen with clopidogrel, but further 80%, 78% and 72%(all p<0.0001) reductions, respectively, were seen with administering eptifibatide. With using the same agonists, fibrinogen binding was significantly reduced, after administering clopidogrel, by 70%, 64% and 81%, respectively(all p<0.0001), and the fibrinogen binding was again further reduced, with administering eptifibatide, by 90%, 95% and 69%, respectively(all p<0.0001). They suggested that the activated GP IIb/IIIa expression and fibrinogen binding findings indicate that eptifibatide provides significant potent antiplatelet activity above that of aspirin and clopidogrel, suggesting additive immediate protection for the treatment of Non-STEMI. The ISAR-REACT 2 trial[90] is a landmark study of better outcomes for Abciximab reducing the risk of adverse events in patients with non-ST-segment elevation ACS and who are undergoing PCI after pretreatment with 600 mg of clopidogrel. Of the 2,022 enrolled patients, 1,012 were assigned to the abciximab group and 1,010 were assigned to the placebo group. The primary end point was reached in 90 patients(8.9%) assigned to abciximab vs. 120 patients(11.9%) assigned to placebo; there was a 25% reduction in risk with administering abciximab(relative risk [RR]: 0.75, 95% CI: 0.58-0.97, p=.03). Among the patients without an elevated troponin level, there was no difference in the incidence of the primary end point events between the abciximab group [23/499 patients (4.6%)] and the placebo group[22/474 patients (4.6%)](RR: 0.99, 95% CI: 0.56-1.76, p=.98), whereas among patients with an elevated troponin level, the incidence of events was significantly lower in the abciximab group[67/513 patients (13.1%)] compared with the placebo group[98/536 patients (18.3%)], which corresponds to an RR of 0.71(95% CI: 0.54-0.95, p=.02) (p=.07 for interaction). These studies provide evidence that additional antiplatelet activity can be achieved when GP IIb/IIIa receptor inhibitors are prescribed on top of an aspirin and clopidogrel regimen.

Additional agents such as pasugrel,[91] AZD 6140[92] and

cangrelor[93] are under clinical investigation and they might be potential alternatives to overcome clopidogrel resistance.

Conclusions

Dual antiplatelet therapy is a cornerstone antiplatelet regimen in the setting of PCI with stenting. Variability or resistance to aspirin or clopidogrel has been demonstrated using various in vivo biomarkers and ex vivo platelet function tests. Accumulating data suggested that patients with resistance are at high risk for ischemic events, including stent thrombosis. Thus, cardiologists have focused much attention on the adequacy of antiplatelet regimens.

Several important issues remain: 1) whether platelet function tests are valid in clinical practice, 2) when, on whom and how to measure the platelet function test, 3) what therapeutic intervention should be done, and 4) whether a platelet function test is cost-effective in clinical settings. If these question or controversies are resolved, then altering therapy based on the results of platelet function tests will be beneficial to our patients.

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