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(Review Article)

Understanding clopidogrel resistance: Mechanisms, detections, implications and surmount the clopidogrel resistant in order to potential solutions

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Abstract

Clopidogrel, a drug belonging to the thienopyridine class of antiplatelet agents, has been utilized either as a standalone medication or in combination with aspirin for averting vascular complications in patients afflicted with atherothrombotic conditions. The selective and irreversible blockade of the P2Y12 receptor by clopidogrel makes it a potent inhibitor of platelet activation and aggregation. In patients with acute coronary syndromes or undergoing percutaneous interventions, combining clopidogrel with aspirin is a crucial approach to antiplatelet therapy. The aim of this review article is to investigate the factors that contribute to clopidogrel resistance, mechanisms involved in clopidogrel resistance, evaluate it through laboratory methods and clinical implications of clopidogrel resistance. Additionally, the article explores the clinical importance of Clopidogrel Resistance and its correlation with harmful cardiovascular events. The paper concludes by proposing potential solutions to this resistance and suggesting the usage of alternative antiplatelet from the new generation.

Keywords: Clopidogrel Resistance; Platelet Aggregation; P2Y12 Receptor; Ticagrelor; “Gold Standard” Method

1. Introduction

The P2Y12 receptor is selectively and irreversibly blocked by Clopidogrel, which effectively inhibits ADP-induced platelet activation and aggregation.1 The CAPRIE study found that the combined use of antiplatelet drugs - clopidogrel and aspirin, was more effective in certain patient groups compared to aspirin alone, due to the distinct mechanism of inhibition of different receptors and pathways.2 In patients with acute coronary syndromes who undergo Percutaneous Intervention (PCI), combined treatment with Clopidogrel and Aspirin is recommended. This combination therapy is considered the standard approach to reducing platelet activation and aggregation during acute coronary syndromes and in patients undergoing stenting. This approach is widely accepted as the "gold standard."3-5 inadequate response to antiplatelet agents is a clinically significant occurrence where cardiovascular events may still happen despite adequate therapy with GpIIb / IIIa inhibitors, Clopidogrel, or aspirin. This phenomenon, also known as resistance or poor responsiveness, has been linked to future cardiovascular events according to various studies, particularly in the case of a deficient response to Clopidogrel.6-13

Despite its significance, understanding of clopidogrel resistance is still lacking, with no clear definition in terms of its pharmacological effect or treatment failure. Additionally, there are no standardized methods to determine its presence and no consensus on alternative treatment strategies for patients with lopidogrel resistance. This review aims to summarize the clinical impact of clopidogrel resistance, its possible mechanisms, and how it can be identified in a clinical setting. It also presents alternative therapeutic options for patients exhibiting or at risk for clopidogrel resistance.5-15

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The use of clopidogrel in current clinical practice:

According to the CAPRIE trial, using 75 mg clopidogrel was slightly more effective than aspirin in preventing secondary events in patients with stroke, myocardial infarction, or peripheral arterial disease .16Additionally, research indicates that clopidogrel is beneficial when combined with aspirin for patients with acute coronary syndromes and those undergoing PCI .17-20 For individuals who cannot take warfarin, clopidogrel may be a viable option in managing atrial fibrillation.21

1.1. Action of Clopidogrel

Clopidogrel is an inactive pro-drug that requires biotransformation within the human body after being orally ingested to attain its antiaggregative properties. The active metabolite responsible for inhibiting platelet aggregation is produced via the cytochrome P450 (CYP)-dependent pathway in the liver. This is contrary to other antiplatelet medication, such as aspirin or ticagrelor, which do not necessitate transformation and are active immediately. Platelet inhibition is achieved through the irreversible binding of P2Y12 receptors, which are activated by adenosine diphosphate (ADP) binding to protein receptors (P2Y1 and P2Y12) on the platelets, leading to ADP-mediated platelet aggregation via the glycoprotein IIb/IIIa (GPIIb/IIIa) complex pathway. Clopidogrel serves as an ADP receptor inhibitor, preventing platelet aggregation, but it does not interfere with arachidonic acid metabolic pathways.22 Several metabolic enzymes, which are encoded by various genes, participate in the complex biotransformation process of clopidogrel.23-25 P-glycoprotein intestinal epithelial cells, which are expressed by the ABCB1 gene, play a crucial role in the absorption of clopidogrel from the gastrointestinal tract.26. The major metabolic pathways in the liver are regulated by the cytochrome P450 2C19 gene (CYP2C19), which is accountable for about 50% of the active metabolites, and the cytochrome P450 3A4 gene (CYP3A4), contributing to 39.8% of active metabolites.27 The P2Y12 receptor and GPIIIa gene-GPIIb/IIIa complex, which are biological targets and effectors of clopidogrel, are both encoded by the P2RY12 gene.28The response to clopidogrel may be influenced by genetic variations and metabolic disturbances occurring at various steps of the complex process.29

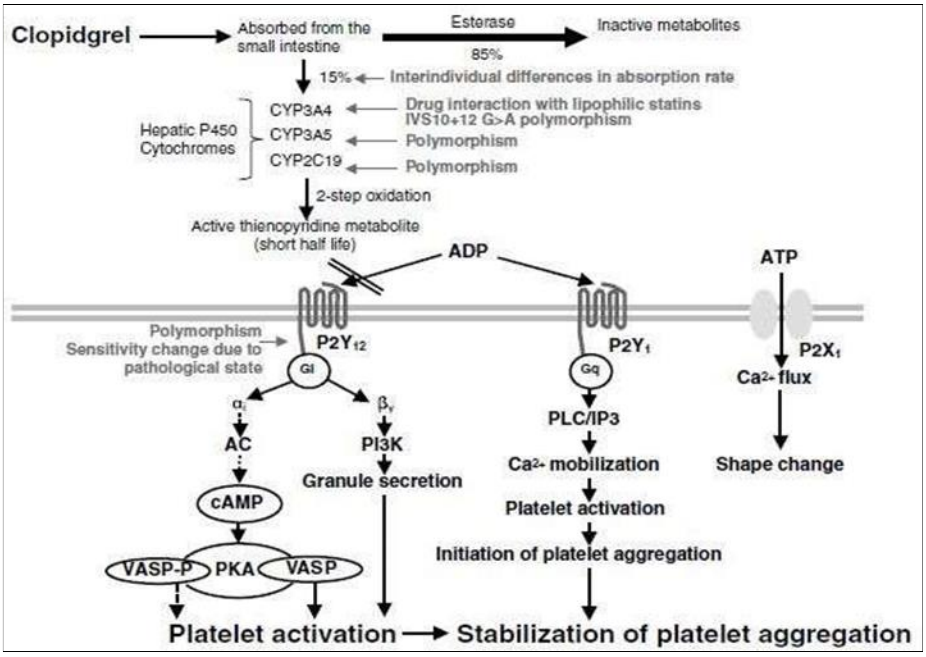

Figure 1 Action of Clopidogrel

Taking the standard dosage of clopidogrel will result in partial P2Y12 several metabolic enzymes, which are encoded by various genes, participate in the complex biotransformation process of clopidogrel. Antagonism, leading to the inhibition of around 50% of ADP-induced platelet aggregation.30 Platelet inhibition caused by clopidogrel is reliant on both the dose and duration of treatment. In healthy individuals, platelet inhibition increases with dosage up to a maximum of 400mg, but there is no additional increase observed with a dosage of 600mg.31 It takes 2 to 5 hours to achieve maximum platelet inhibition with a single 400-mg dose, whereas daily administrations of 75 mg take 3 to 7 days to reach the equivalent level of inhibition.32Clopidogrel not only prevents platelet aggregation but also decreases the expression of proinflammatory markers like CD40 ligand and CD62 P-selectin, which are activated platelet dependent.

Additionally, it reduces the increase in C-reactive protein levels that happen in patients who undergo PCI.33-34CD40 ligand is a strong trigger of vascular inflammation, leading to platelet- leukocyte interactions and tissue factor expression. Clopidogrel can also decrease the creation of platelet-leukocyte conjugates in people with non-ST-segment elevation acute coronary syndrome (ACS).35

1.2. Clopidogrel resistance -Definition

Clopidogrel resistance is characterized by the molecule's inability to inhibit its intended target. The most effective way to demonstrate clopidogrel resistance is by measuring the ADP-induced platelet aggregation before and after treatment, which indicates any residual P2Y12 activity. Platelet activation caused by multiple receptor signaling pathways is responsible for the drug's activity. Consequently, a single treatment approach may not be adequate to overcome clopidogrel resistance.36

1.3. Mechanism of Clopidogrel resistance

The causes behind the uneven response to clopidogrel and its resistance are not fully understood. Variations in intestinal absorption, hepatic metabolism through cytochrome 3A4 (CYP3A4) enzyme, and genetic variations in platelet receptors have been proposed as potential reasons.37-40 In both normal volunteers who received a daily dose of 75 mg and patients undergoing PCI who received loading doses of 300 or 600 mg, ex vivo ADP-induced platelet aggregation showed no more than 30-50% inhibition.41-43 The incomplete blockade of the P2Y12 receptor and inadequate inhibition of ADP- induced platelet aggregation were evidenced by the level of inhibition observed. Studies have repeatedly shown that administering a high loading dose of 600 mg clopidogrel to PCI patients increases inhibition of ex vivo ADP- induced platelet aggregation, reduces nonresponses, and thus suggests insufficient active metabolite generation is a significant contributor to clopidogrel resistance.44-48 Studies conducted lately, which measure the activity of hepatic cytochrome (CYP) P450, propose that differences in the enzyme's activity among individuals are a crucial determining factor.49-51 Lau et al's significant research revealed that the administration of rifampin, which stimulates the activity of hepatic cytochrome P450 (CYP) 3A4, enhances the inhibitory impact of clopidogrel, while substances that contend with clopidogrel for CYP3A4 activity, such as erythromycin, reduce the drug's antiplatelet effect.52-55 Further evidence supporting the crucial involvement of CYP P450 in the formation of the active metabolite of clopidogrel was illustrated in a small trial that involved human subjects. The study evaluated the impact of ketoconazole - a CYP3A4 inhibitor - on the pharmacokinetics and ex vivo platelet inhibitory effects of prasugrel and clopidogrel. Prasugrel is also a thienopyridine that necessitates conversion to an active metabolite via a hepatic cytochrome. The research established that co- administration of ketoconazole did not affect the generation of the prasugrel active metabolite or prasugrel-induced platelet inhibition. In contrast, clopidogrel-induced platelet inhibition was diminished following the administration of a loading dose and also after a maintenance dose.56The decrease in active metabolite generation was observed alongside a reduction of clopidogrel-induced platelet inhibition. Conversely, prasugrel treatment led to better generation of active metabolites, greater platelet inhibition, and a reduced likelihood of nonresponsiveness when compared to treatment with clopidogrel according to another study.

Recent research has shown that the metabolic activation and responsiveness of clopidogrel can be affected by the CYP3A5 and CYP2C19 enzymes. Suh J et al found that individuals with the CYP3A5 expresser genotype had a more significant reaction to clopidogrel compared to those with the non- expresser genotype. Additionally, patients with the non-expresser genotype experiencing stent implantation had poorer outcomes after receiving clopidogrel therapy compared to those with the CYP3A5 expresser genotype.57- 60Two separate studies, conducted by Hulto J et al and Brandt et al respectively, confirmed the impact of the CYP2C19 genotype on the response to clopidogrel in healthy participants.61Ultimately, Angiolillo DJ et al revealed that the IVS10 + 12GNA polymorphism of the CYP3A4 gene could play a significant role in the variability of clopidogrel response.figThe inadequate platelet response to clopidogrel could arise from an elevated quantity of platelet P2Y12 receptors or variations in platelet receptor polymorphism. Studies indicate that genetic polymorphisms in platelet GPIIb/IIIa, GPIa/IIa, or P2Y12 receptors can impact platelet function and potentially influence the variability of clopidogrel response. It has been recently reported that a higher proportion of individuals diagnosed with peripheral arterial disease possess the H2 haplotype of the P2Y12 receptor58, 59.However, another study failed to show a correlation between this haplotype and the responsiveness to clopidogrel. As the connection between genetic variations and how individuals respond to clopidogrel remains uncertain, additional research is necessary to establish a link between receptor variations and the ineffectiveness of clopidogrel. Studies have demonstrated that individuals with diabetes display heightened platelet activation and heightenedresponsiveness to agonists. This escalated platelet reactivity may be associated with the higher occurrence of non-responders and the incidence of ischemic events observed in patients with diabetes. Patients who have a high body mass index (BMI) have also been noted to have a suboptimal platelet response when given the standard 300 mg loading dose. All of the data provided strongly indicate that the main reasons for resistance, as opposed to genetic variations in platelet receptors or intracellular signaling mechanisms, are insufficient metabolite generation due to limitations in intestinal absorption, drug interactions with CYP 3A4, or genetic variations of CYP isoenzymes.62-64 However, these latter mechanisms may be applicable for those patients who continue to exhibit resistance and high platelet reactivity to ADP even after receiving high doses of medications.

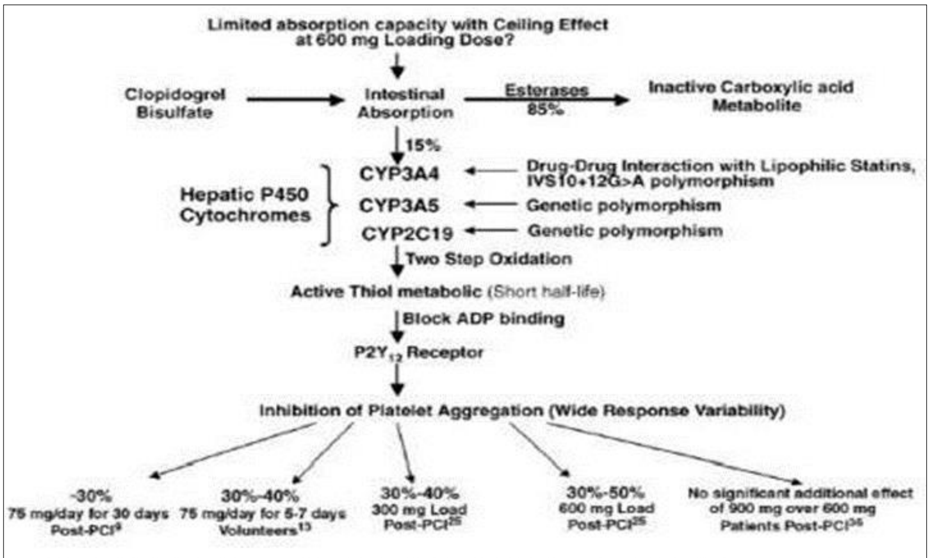

Figure 4 Clopidogrel metabolism and factors affecting clopidogrel responsiveness.

Figure 2 Mechanism of clopidogrel resistance

1.4. Clinical implications of other genetic variations of clopidogrel and response

The association between genetic variants other than CYP2C19 LOF and the risk of recurrent stroke or bleeding in minor stroke or TIA remains uncertain. Approximately 30% of the population in the United States carries the CYP2C19∗17 gene, which enhances CYP2C19 expression. However, it is unclear whether this gene has clinical significance, as some observational studies report a higher risk of bleeding but a lower risk of cardiovascular events, while others do not. The effects of variations in genes that affect the absorption (ABCB1) of clopidogrel and its conversion to its active form (carboxylesterase-1 and paraoxonase-1) also require further exploration, but routine testing for prescribing purposes is not recommended.

Genetic testing offers several advantages, such as the stability of a patient's genotype over time and the ability to conduct tests prior to treatment. Additionally, in patients with LOF alleles, adjustments to the medication type or dosage can be made to modify the risk of vascular events. However, there are drawbacks to genetic testing, including its time-consuming nature, the need for expertise in result interpretation, and the high cost involved. Furthermore, it is not feasible to test for patient compliance.65

1.5. Platelet function testing

When investigating platelet function disorders, a systematic approach is necessary, beginning with collaboration between clinical and laboratory staff to gather a comprehensive medical history. This includes a review of recent and regular medications. laboratory testing is then initated, starting with a complete blood count and a blood film examination if abnormalities in platelet-related parameters are detected by the hematology analyser, such as platelet count, mean platelet volume (MPV), or platelet distribution width (PDW).

In cases where there is a clinical suspicious of platelet defects despite normal results from screening tests, a comprehensive diagnostics workup is crucial.This may involve a panel of specialized tests,including platelet aggregometry, assessment of platelet microRNAs(miRNA),genetic studies, and analysis of signal transduction pathways.

Table 1 provides a summary of the currently available platelet function tests along with their clinical significance.66-70

1.5.1. Platelet aggregometry

Light transmission aggregometry (LTA) is considered the standard method for evaluating platelet function and remains the most commonly used test for detecting and diagnosing platelet function disorders. Platelet-rich plasma (PRP) is mixed in a cuvette positioned between a light source and a detector. Upon addition of various agonists, including collagen, ADP, thrombin, ristocetin, epinephrine, and arachidonic acid, platelet aggregation occurs, leading to an increase in light transmission. Thrombin is a potent stimulator of platelets, but it also induces fibrinogen cleavage and clot formation, making it challenging to use as an agonist for platelet aggregation tests. Instead of thrombin, thrombin receptor activating peptide (TRAP) is utilized to activate thrombin receptors. The platelet aggregation pattern is considered a primary response to an external agonist, followed by a secondary response to the release of dense granule contents. This biphasic response may not be fully visible if high concentrations of agonists are employed. Parameters measured include the aggregation rate or slope (%/min) and the maximum amplitude (%) or percentage of aggregation after a specified duration, typically 6-10 minutes.71-76

Table 1 Major platelet function tests and their clinical applications

Table 1: Major platelet function tests and their clinical applications.

Name of test	Principle	Clinical applications
Platelet aggregometry	Platelet aggregation to a panel of agonists	Diagnosis of inherited and acquired platelet defects
PFA-100/200	High shear platelet adhesion and aggregation	Detection of inherited and acquired platelet defects, monitoring antiplatelet drugs
Flow cytometry	Measurement of platelet GP, secretion, MP, and activation markers by fluorescence	Diagnosis of platelet GP defects, platelet release, PMP, platelet activation markers, monitoring antiplatelet drugs
Impact	Measurement of platelet adhesion and aggregation under high shear	Detection of inherited and acquired platelet defects, monitoring antiplatelet drugs
Thrombelastography (TEG/ROTEM)	Monitoring rate and quality of clot formation	Prediction of surgical bleeding, aid to blood product usage, monitoring antiplatelet drugs
VerifyNow	Platelet aggregation	Monitoring antiplatelet drugs
Multiplate	Platelet aggregation	Monitoring antiplatelet drugs
VASP-P	Flow cytometry with phosphoprotein-phosphorylation	Monitoring P2Y12 receptor activity
Microparticles	Flow cytometry with calibrated beads	Platelet activation markers, intercellular communication

PFA: platelet function analyzer; GP: glycoprotein; MP: microparticles; PMP: platelet-derived microparticles; TEG: thrombelastography; ROTEM: rotational thrombelastometry; VASP-P: phosphorylation of vasodilator-stimulated phosphoprotein-phosphorylation.

The traditional method of LTA remains the most valuable technique for diagnosing a range of platelet abnormalities. However, it has limitations, such as using PRP instead of whole blood under low shear conditions, which does not accurately replicate primary hemostasis due to the absence of red and white cells. Additionally, LTA requires a large sample size, is time-consuming, and is influenced by various preanalytical and analytical factors. Despite published guidelines, the LTA technique lacks standardization. Recent recommendations from the Platelet Physiology Subcommittee of the ISTH SSC offer updated guidance.77-79Other methods, such as whole blood aggregometry or lumiaggregometry, have been introduced as alternatives, but they have not gained widespread use and have not provided additional diagnostic information.

Due to the limitations of the traditional LTA assay, new platelet function tests have been developed that are easier to use.

The platelet function analyzer- (PFA-) 100 and PFA-200 devices (Siemens, Marburg, Germany) are currently the most commonly used tests. They serve as a surrogate for in vitro bleeding time. These devices measure the decrease in flow rate and report the time it takes for full occlusion of the aperture, with a maximum reported time of 300 seconds. This comprehensive platelet function test is convenient, automated, and fast. It simulates several aspects of natural platelet function as it operates in a high shear system and uses whole blood instead of PRP. Recently, the clinical applications of these tests have been reviewed, including screening for von Willebrand disease (vWD) and monitoring its treatment, identifying inherited and acquired platelet abnormalities, monitoring antiplatelet therapy, and evaluating the risk of surgical bleeding.80-81Nevertheless, the CT is impacted by platelet count and hematocrit, leading to a lack of specificity for any specific disorder. As a result, although normal PFA CT results can reasonably rule out severe vWD or platelet dysfunction, they may not rule out the possibility of a mild vWF deficiency or platelet disorder. Therefore, if an abnormal CT suggests a potential platelet function defect, it must be confirmed by more targeted and accurate tests.

1.6. Flow cytometry

Whole blood flow cytometry is a potent and widely` utilized laboratory technique for evaluating platelet function and activation. Despite its need for complex equipment, availability of monoclonal antibodies, and lack of standardization, it offers several advantages such as requiring only a small volume of whole blood and being unaffected by platelet count. The commonly employed flow cytometry tests include assessing the baseline status of platelet GP receptors and analyzing the composition of platelet granules. Flow cytometry can be employed for quantifying deficiencies in GP IIb/IIIa in Glanzmann's thrombasthenia and GP Ib/IX/V in Bernard- Soulier syndrome. Furthermore, it has been developed to measure dense granules through mepacrine uptake and release.70,Flow cytometry enables the evaluation of individual platelet functionality and the determination of platelet activation markers expression on individual platelets, as well as the quantification of interactions between platelets and other blood cells.

P-selectin expression on the surface of platelets (indicative of alpha-granule secretion), the activation-induced conformational change of GP IIb/IIIa (detected using monoclonal antibody PAC-1), platelet- leukocyte complexes, examination of microparticles, exposure of negatively charged anionic phospholipids on the platelet surface (procoagulant activity), and phosphorylation of vasodilator-stimulated phosphoprotein (VASP) as an indicator of P2Y12 receptor activation- dependent signaling are the most commonly evaluated platelet activation markers by means of flow cytometry. (Bio-Cytex, Marseille, France).82-84Numerous labs have conducted measurements on various platelet activation markers and demonstrated their increased levels in different clinical scenarios including unstable angina, acute myocardial infarction, preeclampsia, peripheral vascular disease, and cerebrovascular ischemia.

Other point –of-care testing (POCT): Platelet function tests are being increasingly suggested as perioperative tools to assist in predicting bleeding or for monitoring the effectiveness of different prohemostatic therapies. With more patients receiving antiplatelet drugs, the risk of bleeding is also rising. Though traditional LTA still plays a vital role in assessing platelet disorders, it is not easily feasible in acute care settings. The growing demand in clinical practice, along with the advancement of simpler POCT machines, has led to platelet function tests being conducted outside specialized clinical hemostasis labs. 70,83,84The impact cone was initially developed to monitor the ability of platelets to adhere to a polystyrene plate. The device includes a microscope and conducts staining and image analysis of the platelets that stick together and form aggregates at a high shear rate of 1,800/sec. The results are documented as the proportion of the platelet-covered surface area (surface coverage) and the average size of the attached particles. The adhesion process relies on the presence of von Willebrand factor (vWF), binding between fibrinogen and platelets GP Ib and IIb/IIIa. The assay is fully automated, uncomplicated, and quick to perform, utilizing a small sample of whole blood. Emerging evidence suggests that the impact cone has the potential to identify various platelet abnormalities and von Willebrand disease (vWD), and it might also serve as a screening tool. However, the fully automated version of the impact cone has limited practical application, thereby necessitating further research.70,82,83TEG and ROTEM, manufactured by Pentapharm GmbH, Munich, Germany, utilize an oscillating cup to analyze the physical characteristics of clot formation in whole blood samples. TEG provides a comprehensive assessment of factors such as fibrin formation, clot growth, clot strength, clot stability, and fibrinolysis. One of the key advantages of TEG/ROTEM is their ability to provide a holistic overview of clot formation and facilitate the interaction between different components of whole blood, including platelets and the coagulation system. These instruments have traditionally been employed in surgical and anesthesiology departments to assess bleeding risk and guide transfusion strategies. Nonetheless, there is variability between different laboratories in their results, and the analysis itself is time- consuming, taking at least 30 minutes.71-74,85,86

1.7. Management of clopidogrel resistance

Given the information presented regarding the mechanisms involved in clopidogrel resistance in that the frequency of CYP enzyme polymorphisms, it appears that the primary reason for clopidogrel resistance is a decrease in the availability of the active metabolite. As a result, is there a possibility to address or overcome clopidogrel resistance?

1.7.1. Higher dose

One possibility could be to consider increasing the dosage of clopidogrel. Typically, in clinical practice, a loading dose of 300 mg is often prescribed. Two studies have evaluated the effectiveness of a higher loading dose of 600 mg. The study conducted by Cuisset et al. examined this particular dosage.87In a study conducted by L'Allier et al., a total of 292 patients who underwent stenting for non- STEMI were randomly assigned to receive either a loading dose of 300 mg or 600 mg of clopidogrel at least 12 hours before undergoing PCI. In addition, all patients were prescribed a daily dose of 75 mg clopidogrel along with 160 mg of aspirin for one month following the intervention. The study found that the high-loading dose group exhibited significantly lower levels of ADP-induced platelet aggregation and expression of P- selectin compared to the low-loading dose group. Furthermore, during the one-month follow-up period, the occurrence of cardiovascular events was significantly lower in the high-dose group (7 events) compared to the low-dose group (18 events), with a p-value of 0.02. This finding remained consistent even after adjusting for traditional cardiovascular risk factors, as indicated by a p-value of 0.035.88In a pilot study conducted by Angiolillo et al., a total of 148 patients undergoing elective PCI were randomly assigned to three different treatment groups. Group A received a clopidogrel dose of 300 mg the day before the procedure, plus 75 mg in the morning of the procedure. Group B received a clopidogrel dose of 600 mg in the morning of the procedure. Group C received a clopidogrel dose of 600 mg the day before the procedure, plus 600 mg in the morning of the procedure. The study found that the degree of inhibition of platelet aggregation caused by ADP was significantly higher in Group C compared to Groups A and B. This indicates that the use of a double bolus dose of clopidogrel at 600 mg per dose achieved higher levels of platelet inhibition compared to the conventional single loading doses.89The authors assessed the effectiveness of a higher daily maintenance dose of clopidogrel (150 mg) compared to the recommended dose (75 mg) in patients undergoing elective PCI. Both groups received the respective doses for 30 days, after which they switched back to standard dosing. Importantly, patients receiving 150 mg clopidogrel/day had lower ADP-induced (20 μM) platelet aggregation compared to those receiving 75 mg clopidogrel/day (52.1% vs. 64.0%; P < 0.001). A similar pattern was observed in a study involving 60 patients.90In the study conducted by Bonello et al., patients undergoing elective PCI were given a 600 mg loading dose of clopidogrel within 12 hours prior to the procedure, followed by either 75 mg or 150 mg as a maintenance dose for the next 30 days. The results showed that patients receiving the higher dose of 150 mg had significantly better platelet aggregation in response to 5 μM ADP compared to those receiving the lower dose of 75 mg (45.1% vs. 65.3%; P < 0.001). Similarly, platelet function inhibition as measured by the verify Now assay was also significantly better in the 150 mg group compared to the 75 mg group (60.0 vs. 117.0 P2Y12 reaction units; P = 0.004). Other studies have also investigated the impact of higher doses of clopidogrel on platelet reactivity in patients who are resistant to the effects of clopidogrel.91162 patients who had a VASP phosphorylation index greater than 50% after receiving a 600-mg loading dose of clopidogrel were randomly assigned to either a control group or a VASP-guided group. In the VASP-guided group, patients received additional bolus doses of clopidogrel to reduce their VASP index to below 50%. Notably, dose adjustment was successful in 67 out of 78 patients in the VASP-guided group, resulting in a significant decrease in the VASP index from 69.3 to 37.6 (P < 0.001). Twenty-six patients required four doses, but these were ineffective in 11 patients. Interestingly, the VASP-guided group had a significantly lower rate of major adverse cardiac events over the course of one month compared to the control group (0% vs. 10%; P = 0.007), and there was no difference in the rate of major or minor bleeding between the two groups (total: 5% vs. 4%; P = 1). Angiolillo et al. conducted this study.92In a study involving patients who did not respond adequately to a daily dose of 75 mg of clopidogrel (with platelet inhibition below 50%), a higher maintenance dose of 150 mg/day was administered for a duration of one month (n = 17). The study revealed a significant increase in platelet inhibition measured using the Verify Now P2Y12 assay, from 27.1% to 40.6% (P = 0.009 when compared to a control group). However, it is important to note that only 35% of patients achieved a platelet inhibition level of equal to or greater than 50%. Similar results were observed in a larger-scale study.93A total o 153 patients with inadequate response to clopidogrel (platelet reactivity index ≥69%) were randomly assigned to receive either a daily dose of 150 mg (n = 58) or 75 mg (n = 95) of clopidogrel. Following two weeks, the group receiving 150 mg/day of clopidogrel exhibited significantly reduced platelet reactivity index compared to the group receiving 75 mg/day (43.9% vs. 58.6%; P < 0.001), resulting in fewer individuals who did not respond to treatment (8.6% vs. 44.7%; P = 0.004). It should be noted that among the patients in the 75 mg/day group, 20 out of 31 individuals achieved a positive response (i.e., platelet reactivity <69%) after switching to 150 mg/day of clopidogrel for two weeks.

Together, these studies demonstrate the potential use of higher initial and/or ongoing doses of clopidogrel. However, the effectiveness of increasing the dose of clopidogrel had limited benefits, and many patients still did not respond adequately to the medication. This was further supported by a series of cases reported by Pena et al.94Out of the seven patients included, four patients showed resistance to a dosage of 225 mg/day, and two of them remained resistant even after the dosage was increased to 300 mg/day.

In addition, the studies did not evaluate the potential long-term consequences of administering elevated doses of clopidogrel, which may be required for patients with clopidogrel resistance in clinical practice.

1.8. Alternative agents: Prasugrel

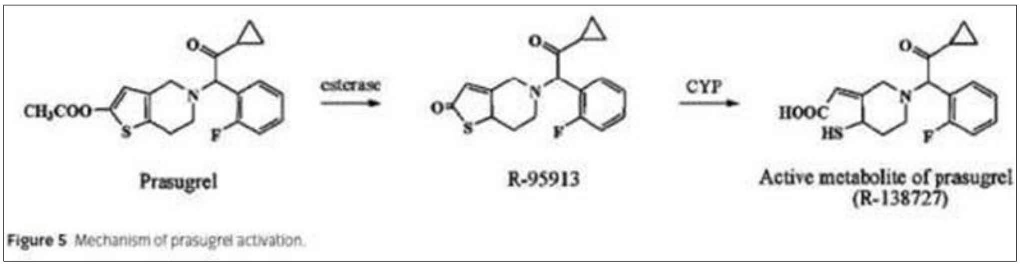

Figure 3 Metabolism of Prasugrel

Prasugrel is a new anticoagulant drug that, similar to clopidogrel, acts on the ADP receptor in platelets. Esterase are responsible for generating the deacetylated form of prasugrel (as shown in Figure 5). This hydrolysis process happens quickly both in laboratory settings and within the body, resulting in undetectable levels of circulating prasugrel soon after it is administered.95-96In the PRINCIPLE-TIMI 44 study, the efficacy of prasugrel was compared to that of clopidogrel.

Similar to the TRITON-TIMI-38 study, this trial involved 13,608 patients with moderate-to- high-risk ACS who were scheduled to undergo PCI. Patients were randomly assigned to receive either a 60-mg loading dose of prasugrel followed by a 10-mg daily maintenance dose or a 300-mg loading dose of clopidogrel followed by a 75-mg daily maintenance dose for 6-15 months. Results from the study showed that the primary endpoint (defined as death from cardiovascular causes or nonfatal MI or stroke) occurred in significantly fewer patients treated with prasugrel compared to those treated with clopidogrel (9.9% vs. 12.1%, respectively; HR = 0.81; 95%CI = 0.73-0.90; $P < 0.001$). Moreover,

prasugrel was associated with significantly lower rates of MI, urgent target-vessel revascularization, and stent thrombosis compared to clopidogrel. However, it is important to note that prasugrel was also associated with an increased risk of major bleeding and life-threatening bleeding in this study.97-98A total of 201 participants were administered either a 60-mg loading dose of prasugrel or a 600-mg loading dose of clopidogrel to evaluate their effects on platelet aggregation and aggregation-thrombolysis in MI. The primary objective of this study was to measure the inhibition of platelet aggregation (in response to 20 μmol/L ADP) after the loading-dose phase. The results showed a significant difference in favour of prasugrel, with a higher rate of platelet aggregation inhibition compared to clopidogrel (61.3% vs. 46.1%; $P < 0.001$).99Hence, prasugrel might be more suitable for patients who are using PPIs. It is interesting to note that in the study conducted by Pena et al. 100, as mentioned earlier, four out of the seven patients were transitioned to prasugrel, which resulted in a significant improvement in platelet aggregation for these individuals. The authors attributed these findings to the presence of heterozygous or homozygous ∗2 polymorphisms in those patients. Clearly, further investigations are required to examine the advantages of adjusting treatment based on prospective polymorphism testing or when clopidogrel resistance arises.

1.9. Cilostazole

Cilostazol hinders platelet aggregation by counteracting the activity of phosphodiesterase 3 and consequently inhibits the degradation of cAMP in platelets. It has been theorized that cilostazol could provide an alternative approach for patients with inadequate responses to clopidogrel. However, in numerous countries, cilostazol is only approved for treating intermittent claudication; in Japan and other Asian countries, it is also approved for preventing recurrent cerebral infarction.

Nonetheless, clinical studies have demonstrated significant benefits of using cilostazol in combination with clopidogrel and aspirin to reduce restenosis in patients undergoing modern stent-based percutaneous interventions101, as well as to decrease adverse cardiac and cerebral events.102 Therefore, cilostazol may be more effective than administering high maintenance doses of clopidogrel .103However, these studies observed that cilostazol was associated with a higher frequency of discontinuation due to adverse events compared to clopidogrel or aspirin.

1.10. Ticagrelor

Ticagrelor is a novel platelet aggregation inhibitor that has recently completed Phase III studies, showing promising results. Similar to clopidogrel, ticagrelor binds to P2Y12 to block the ADP receptor on platelets, thereby inhibiting platelet aggregation. However, unlike clopidogrel, ticagrelor binds reversibly to P2Y12 and does not displace ADP from the receptor, specifically targeting the signaling induced by 2-MeS-ADP.104Furthermore, ticagrelor does not require hepatic enzymatic activation, suggesting that it may provide more consistent platelet inhibition with a reduced risk of drug interactions and is not influenced by genetic variations in the CYP system.105 In the platelet inhibition and patient Outcomes (PLATO) study, 18,624 patients hospitalized with acute coronary syndrome were randomly assigned to receive either ticagrelor (180-mg loading dose followed by 90 mg twice daily) or clopidogrel (300-600 mg loading dose followed by 75 mg daily). After 12 months, the primary outcome (a combination of death from vascular causes, myocardial infarction, or stroke) occurred in 9.8% of patients treated with ticagrelor compared to 11.8% of those treated with clopidogrel (HR = 0.84; 95%CI = 0.77-0.92; P < 0.001). However,the PLATO study found that ticagrelor was associated with higher rates of ventricular pauses and dyspnea, although the exact cause has not been determined.106

2. Conclusion

Resistance to clopidogrel is a prevalent clinical condition with potential serious consequences. It heightens the mortality risk and other unfavorable outcomes following cardiovascular interventions due to inadequate suppression of platelet aggregation in affected individuals. Patients who take medications that undergometabolism via the cytochrome P450 system, like statins and PPIs, as well as those with genetic variations in these enzymes, particularly CYP2C19, are prone to displaying suboptimal responses to clopidogrel. Such patients might benefit from higher initial doses of clopidogrel, combination therapy involving cilostazol along with clopidogrel and aspirin, or transitioning to alternative medications such as prasugrel, ticagrelor, or cilostazol. In situations where genetic screening is unavailable, the use of flow cytometry or point-of-care devices to prospectively identify patients at risk of clopidogrel resistance may aid in prescribing clopidogrel or alternative treatments. Nevertheless, further research is necessary to validate the clinical effectiveness of this approach. Additionally, while most studies investigating clopidogrel resistance have primarily focused on patients with acute MI, it is important to note that clopidogrel is also commonly used in individuals with other conditions like cerebrovascular disease and peripheral artery disease (PAD). Consequently, conducting studies to assess the clinical impact of clopidogrel resistance in patients with cerebrovascular disease or PAD would provide insight into the potential risks of severe adverse events in these patient populations.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to disclosed.

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