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REVIEW

The value of using polymorphisms in anti-platelet therapy

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OBJECTIVES and BACKGROUNDS: Cardiovascular events occure as a result of various risk factors, such as uric acid (UA), inflammation, hormones and other materials that induce C- reactive protein (CRP) expression. These factors lead to complement activation, and endothelial damages. Damaged endothelial cells release heparan sulfate which inhibits tissue factor activity and von Willed brand factor (VWVF) and causes aggregation. Finally this cascade of events cause platelets aggregation and leads to heart ischemia and cardiovascular events.

DISCUSSION: Anti-platelet therapy is an interesting premise. Anti-platelet resistance patients and bleeding as a result of using ticagrelor and prasugrel should be considered in this treatment methods. Anti-platelet drugs such as clopidogrel are prescribed in cardiovascular events. Platelets have VWF receptors and P2Y12 receptors on their surface, and thus, targeting these receptors can be useful in treatment. The active metabolites of clopidogrel bind to P2Y12R and inhibit ADP binding; thus, clopidogrel inhibits aggregation by interfering in several events as a result of the inhibition of ADP attachment to P2Y12R of the platelet. However, the polymorphisms of P2Y12 and other genes mentioned in Table 1 showed treatment resistance in anti-platelet therapy, highlighting that these SNPs can be helpful in anti-platelet therapy.

CONCLUSION: The knowledge of these SNPs may decrease the number of unwanted effects that endanger patients with cardiovascular diseases and avoids ineffective anti-platelet therapy in several patients. Clopidogrel, ticagrelor, prasugrel, and aspirin and CYP2C19 and their SNPs are very important subjects in anti-platelet therapy. To present the importance of using pharmacogenetics in anti-platelet therapy, we discuss here the association between these drugs and the SNPs for therapeutic resistance.

Keywords cardiovascular events, clopidogrel, CYP2C19 polymorphisms, anti-platelet drugs

Introduction

Cardiovascular events, which interfere in the correct blood flow in vessels of the heart, commonly lead to ischemia and death (Jian et al., 2016). Many risk factors, including cholesterol, uric acid, S100 proteins, inflammations, and hormones, such as adrenalin, have been indicated as the stimuli for cardiovascular events and heart ischemia (Donato et al., 2013; Andreadou et al., 2017). A number of these stimuli, for example uric acid and inflammation, increase the expression of CRP, which causes complement activation. Complement mediators damage the endothelial lining (Gorsuch et al., 2012). Genetic mutations, which cause structural changes in epithelial lipids and receptors, can be presented as predictive risk factors for cardiovascular disease and ischemia (Razzaghi et al., 2013). Genes and SNPs affect the treatment responses and the prevalence of cardiovascular events (Qiu et al., 2015). Damaged endothelial cells release VWF and heparan sulfate (Spiel et al., 2008; Nadir, 2014). These events ultimately led to aggregation and cause cardiovascular events and they can culminate in necrosis ischemia, which was a significant problem in heart ischemia (Wang et al., 2016). The use of anti-platelet drugs, such as clopidogrel, aspirin, prasugrel, and ticagrelor, is a treatment approach for cardiovascular and ischemic events, but approximately 45% of people are thought to be clopidogrel-resistant and may have a considerable number of polymorphisms (Ray, 2014). Aspirin resistance is also fairly prevalent (Cui et al., 2015). Genes such as CYP2C19 and ABCB1 can help in the estimation of the treatment responses (Martínez-Quintana and Tugores, 2015). The identification of resistance polymorphisms in anti-platelet therapy is useful to increase the accuracy of the diagnosis and treatment efficacy (Farthing et al., 2015). In addition, the use of a pharmacogenetic

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approach to anti-platelet therapy can provide a balance in anti-platelet therapy to decrease the chance of bleeding outcomes as a result of anti-platelet drugs such as prasugrel and ticagrelor (Malhotra et al., 2015). At present, the pharmacogenetic tests are not used in clinics (Yip and Pirmohamed, 2013). In this review, we have briefly discussed cardiovascular events, because of their prevalence and the importance of using anti-platelet drugs in their treatment, and we have discussed anti-platelet drugs and anti-platelet resistance SNPs to offer insight into the combination of anti-platelet drugs and genetics to improve treatment safety, especially in patients with cardiovascular events. We also suggested the use of pharmacogenetics, especially for patients who are anti-platelet-resistant, in order to increase the treatment efficacy.

Cardiovascular events at a glance

An increase in serum UA or other stimuli can result in oxidative stress (Tekin et al., 2013), which can lead to endothelial dysfunction and inflammation and affect platelet function (Kosacka et al., 2015). Alternatively, the stimulus can induce CRP expression, which can cause complement activation, produce pro-inflammatory mediators, and damage the endothelium (Castellano et al., 2010). Endothelial damages led to the development of organ dysfunction (Bro-Jeppesen et al., 2016). As a result of endothelial damage, two major events emerged: first, VWF, which plays an important role in platelet adhesion and aggregation, is released from endothelial cell walls (Andersson et al., 2012); second, there is a decrease in heparan sulfate on the epithelial surface (Nadir, 2014). The mechanism of the anti-platelet drug effect in cardiovascular events is illustrated in Fig. 1. Finally, platelets attach to the endothelial cell walls and form plaques. Plaques lead to cardiovascular events and finally cause necrosis ischemia, which is a significant problem in many patients with heart ischemia (Wang et al., 2016). Myocardial necrosis is indicated by lactate dehydrogenase (LDH), aspartate amino transferase (AST), creatinine kinase (CK), CK isoenzyme-MB (CK-MB), and the troponin test (Lippi et al., 2013). The increased VWF plasma level results from different stimuli, including hypoxia, inflammatory cytokines, and adrenalin. The VWF serum level is significantly associated with cardiovascular events and VWF is a marker and a mediator in these events (Spiel et al., 2008). VWF receptors on platelets cause platelet aggregation, which can lead to cardiovascular events and ischemia (Stone et al., 2013). VWF is cardiovascular disease progression marker of considerable value (Men et al., 2015). Heparan sulfate is a tissue factor inhibitor; heparan sulfate binds to tissue factor and inhibits its activation (Nadir, 2014). Thus, an increase in the serum level of heparan sulfate as a result of a decrease on the endothelial cell walls results in an increased probability of aggregation. Thus, VWF and heparan sulfate are valuable factors for the estimation of ischemia and cardiovascular events.

Anti-platelet therapy

Anti-platelet drugs, such as clopidogrel and aspirin, are used to disrupt plaques and decrease thrombotic aggregations in patients with cardiovascular disease (Kaikita et al., 2014). Patients who have at least one reduced function cytochrome P450 2C19 (CYP2C19) allele show resistance to clopidogrel (Kaikita et al., 2014). In contrast, hemorrhage is an important event in anti-platelet therapy, so VWF should be monitored (Jahn et al., 2017). Prasugrel and ticagrelor appear to increase non-coronary artery bypass graft (CABG) major bleeding (Malhotra et al., 2015). Therefore, the platelet function assays are useful tests (Lowe and Rumley, 2014). Activated platelets release ADP, which affects the platelet P2Y12 receptor (P2Y12R) and has an important effect in aggregation. Thus, P2Y12R polymorphisms should be observed in response to P2Y12 inhibitor therapies, especially in cardiovascular disease (Thomas and Lip, 2017). Platelet function, genetic tests, and drug-drug interactions should be considered in anti-platelet therapy (Rollini et al., 2017). The mechanism of anti-platelet therapy is illustrated in Fig. 1.

Clopidogrel at a glance

Clopidogrel is an anti-platelet drug; its metabolites are P2Y12 ADP receptor antagonists (Tough et al., 2016). Clopidogrel requires bioactivation to produce its metabolites. The bioactivation mechanism occurs in the cytochrome P450 (CYP450) system of liver mitochondria (Sen et al., 2015). Genes such as CYP2C19 encode the cytochrome enzyme and correlate to clopidogrel response through the transformation of clopidogrel to its active metabolite (Gajda et al., 2014). In the CYP450 system, an esterase converts clopidogrel to carboxylic acid metabolites that are P2Y12 antagonists. This esterase is encoded by the paraoxonase 1 (PON1) gene (Ou et al.,2016). The Rs662 polymorphism in PON-1 gene is associated with clinical outcomes: this SNP is linked to clopidogrel bioactivation, but is not associated with clopidogrel response variability (Scott et al., 2011). The metabolites bind to P2Y12 receptor on the platelet surface and decrease its expression; that is, clopidogrel active metabolites cause P2Y12R alteration and inhibit adenylate cyclase (AC) inactivation and these process lead to the activation of the ADP-dependent IIb/IIIa glycoprotein complex (GPIIIb/IIa) (Martínez-Quintana and Tugores, 2015; Bozzi et al., 2016). This is a two-step process; first, clopidogrel must react with hepatic CYP450 to be converted to its active metabolites and then the active metabolites react as a thiol reagent and bind to G-protein-coupled P2Y12R on the platelet. Finally, clopidogrel stops the binding of ADP to P2Y12R on the platelets (Gajda et al., 2014; Martínez‐Quintana, and

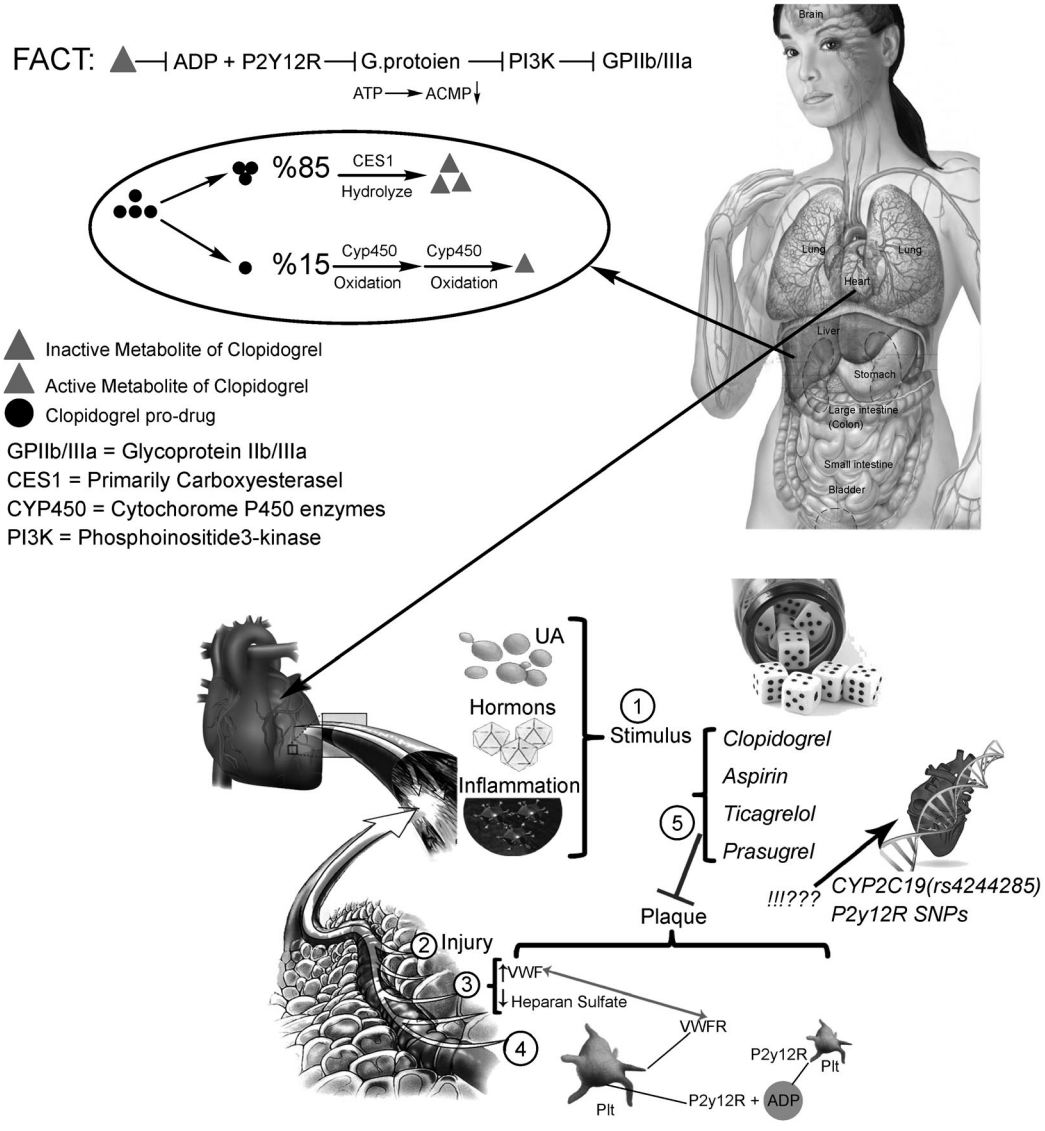

Figure 1 Uric acid, inflammation, hormones, and other stimuli damage endothelial cells. VWF increased on the damaged endothelial surface, but heparan sulfate decreased. These changes in the epithelial surface cause platelet adherence, aggregation, and ultimately plaque formation. Anti-platelet drugs disrupt the plaques; these drugs produce metabolites that inhibit platelet activation by attaching to P2Y12R. Clopidogrel is a pro-drug that reacts with CYP450 to be transformed to its active metabolite. The metabolites then bind to P2Y12R and inhibit G-protein (GP), which leads to PI3K inhibition and decreased GP expression on the platelet surface. GP mediates fibrinogen binding to platelets and causes aggregation, adherence, and thrombus formation. Abbreviations: UA, uric acid; VWF, Von Willebrand Factor; ADP, Adenosine di-phosphate; PI3K, phosphoinositide3-kinase; CES, primarily carboxylesterase 1; GP, Glycoprotein; CYP450, cytochrome P450 enzymes.

Tugores, 2015). Briefly, the clopidogrel active metabolites inhibit ADP attachment to P2Y12 receptor, which inhibits aggregation and reduces cardiovascular events in patients who are at risk) (see Fig. 1). The genes that encode the P2Y12 receptor, CYP2C19 and PON1, should also be considered in relation to clopidogrel. CYP2C19*2 allele carriers have higher platelet reactivity (PR), which can lead to cardiovascular events. Thirty percent of Caucasians and 50% of East Asians appear to have this allele (Gajda et al., 2014); and 85% of this population has a loss of function allele present as the rs4244285 variant (Daly and Becker, 2014). This loss of function causes the production of CYP450 enzymes that are inefficient in oxidation (Perry and Shuldiner, 2013). CYP2C19*2 carriers have a 35% higher risk for major adverse cardiovascular events (MACE) in patients whether they are homozygous or heterozygous (Scott et al., 2011). In CYP2C19*3, the rs4986893 allele frequency in Asia is 2%–9% and this SNP can cause clopidogrel resistance too (Shuldiner et al., 2014). The ATP binding cassette subfamily B member 1 (ABCB1) gene rs1045642 is associated with a decrease in clopidogrel active metabolites (Perry and Shuldiner, 2013). ABCB1 encodes the ATP-dependent drug

efflux pump, which affects the gastrointestinal absorption of clopidogrel and the impairment of this protein can lead to clopidogrel resistance (Hurst et al., 2013). SNPs and mutations in the indicated genes appear to be the cause of treatment resistance (Yi et al., 2017) and there is variation in its prevalence in different populations worldwide (Chan et al., 2014; Yi et al., 2016). We have categorized these SNPs in Table 1. SNPs that affect the treatment response to clopidogrel do not affect the response to ticagrelor and prasugrel; therefore, we can consider prasugrel and ticagrelor, which have no common genetic influence with clopidogrel, but a number of studies showed both these drugs increased bleeding in patients compared with clopidogrel (Cui et al., 2015; Golukhova et al., 2015). Recent studies have investigated clopidogrel reversal owing to increased amounts of VWF in the patient's blood (Jahn et al., 2017). Furthermore, GPIIb/IIIa can bind to fibrinogen and cause thrombus formation (Sumaya and Storey, 2017). Therefore, physicians should consider these complexities in their treatment decisions.

Ticagrelor at a glance

Ticagrelor is a direct acting inhibitor, which acts faster than clopidogrel and inhibits P2Y12R more consistently (Capodanno et al., 2010). Additionally, ticagrelor decreased cardiovascular events, but significantly increased the bleeding rate in comparison with clopidogrel (Xin et al., 2017). Several studies claim that more patients are treated with ticagrelor (Xin et al., 2017). In comparison with clopidogrel, there were no differences in hemorrhagic events (Jiménez-Brítez et al., 2017) and ticagrelor was not influenced by CYP2C19 variants (Friede et al., 2017). It has been shown that ticagrelor is also more effective in patients with atherosclerotic disease in comparison to aspirin (Amarenco et al., 2017). In addition, ticagrelor incompletely inhibits Thromboxane A2 formation and was reported to be synergetic with the function of aspirin rather than prasugrel (Armstrong et al., 2017). It is hypothesized that familiarity with the knowledge that clopidogrel reduces cardiovascular events caused physicians to prescribe clopidogrel, but the increased use of ticagrelor and prasugrel might be associated with a decrease in acute coronary syndrome (ACS) and death in comparison with clopidogrel (Sheikh Rezaei et al., 2017).

Prasugrel at a glance

Prasugrel is another anti-platelet drug that shows less thrombus plaque in patients with ACS in comparison with clopidogrel (Kubo et al., 2017). Prasugrel is an irreversible antagonist of P2Y12R. Prasugrel is converted to its active metabolite by the hepatic CYP system, similar to clopidogrel, but the active metabolites of prasugrel are more effective than clopidogrel (Angiolillo et al., 2008). It has less risk of thrombosis, but a higher risk of bleeding in comparison with ticagrelor (Sharma and Mascarenhas, 2017). Thus, prasugrel or ticagrelor might overcome the issue of clopidogrel resistance and the need for genotyping, but we should pay attention to the risk of bleeding in the patient (Fontana et al., 2013). Severe bleeding and steady rate of ischemic events in sensitive users of prasugrel have persuaded physicians from Europe and the United States to develop guidelines for the treatment process (Bonaca and Wiviott, 2016). Several studies have shown that combined use of prasugrel, ticagrelor, and aspirin should be applied cautiously as it may lead to significantly more bleeding in comparison with clopidogrel (Verlinden et al., 2017).

Aspirin at a glance

Aspirin is a salicylate drug with an anti-platelet effect that results from the inhibition of thromboxane A production (Majumdar et al., 2014). Aspirin acetylates the hydroxyl group of cyclooxygenase (COX)-1 and (COX-2). Acetylated

Table 1 Notable SNPs in anti-platelet therapy

Gene	SNP	Treatment response	Other effects	Ref.
P2Y12	rs6809699 rs17602729	Clopidogrel use appears be dangerous	Clopidogrel results in four times more ICVD than for wild type	Sen et al., 2015
P2Y12R	rs2046934 rs3732759 rs6798347 rs6787801 rs6701273 rs6785930	Clopidogrel resistance	Might be associated with CAD - - -	Yang et al., 2016 Nie et al., 2017
CYP2C19	rs776746 rs4244285	Clopidogrel resistance	-	Yi et al., 2016
CYP2C19*2 CYP2C19*3	rs4244285 rs4986893	-	Asian populations have a higher rate of cardiovascular events	Qiu et al., 2015
TBXA2R	rs5758	Aspirin resistance	-	Cui et al.,2015
GNB3	rs5445	Aspirin resistance	-	Cui et al.,2015

Abbreviations: ICVD, Ischemic cerebrovascular disease; CAD, coronary artery disease.

COX1/2 decreases the conversion of arachidonic acid to prostaglandin H2 (PGH2). Finally, PGH2 is converted to prostanoids such as thromboxane A2 (TXA2) and prostaglandins such as prostaglandin E2 (PGE2). Platelets express COX-1, which is affected by a low dose of aspirin. At a high dose, aspirin affects PGH2 and inhibits aggregation mechanisms (Thomas and Storey, 2014). Aspirin and clopidogrel are prescribed for ischemic cardiovascular events and precutaneous coronary intervention (PCI) (Bozzi et al., 2016). Five polymorphisms of glycoproteins associated with aspirin resistance have been investigated (Cohen and Downey, 2014; Rao et al., 2016). PEAR1 and CES1 genes show variable response in clopidogrel and aspirin therapy (Bozzi et al., 2016). According to the arachidonic acid test (AA), which is not specific, the prevalence of aspirin resistance is higher than the observed prevalence from more specific tests (e.g., thromboxane B2 metabolites) (Olechowski et al., 2017). Thus, it is important to develop a more specific and reliable test to diagnosis patients with aspirin resistance.

Predominant polymorphisms in anti-platelet therapy

The variations in response to anti-platelet drugs occurs as result of the different SNPs in P2Y12R (Nie et al., 2017) and the CYP2C19 enzyme, which is located at chromosome 10 and causes variation in the hepatic expression of the enzyme (Cavallari and Obeng, 2017). Additionally, between 5.5% and 60% of the population has been reported as aspirin-resistant. This ambiguity appears to result from the absence of a standard definition of aspirin response (Majumdar et al., 2014), the prevalence of patients with anti-platelet resistance, and the different reaction in their body, which was interesting from a pharmacogenetic viewpoint. We tried to categorize the number of considerable polymorphisms in Table 1 to provide better information on patients with anti-platelet resistance and the notable SNPs, which cause different reactions.

Conclusion and future perspectives

In recent years, anti-platelet therapy has been used worldwide and anti-platelet therapy-resistant polymorphisms have become a subject of interest. New findings identified that genes and polymorphisms were mainly responsible for anti-platelet resistance. According to the mechanism of anti-platelet drugs, investigation into the genes that are involved in CYP450 expression may be helpful in clopidogrel therapy. Genes such as CYP2C19 and its polymorphisms, especially rs4244285, should be considered in clopidogrel therapy and physicians should be cautious of this gene. Additionally, the P2Y12 variants rs17602729 and rs6809699 must be evaluated in clopidogrel therapy because they can lead to ICVD. Thus, the use of genetics in association with anti-platelet therapy is essential to avoid extra costs of treatment and to decrease the risk of death and complications related to the genetic background of the patient. In contrast, ticagrelor and prasugrel can cause bleeding in patients with cardiovascular events, so their use requires special evaluation. The use of coagulation factors, such as VWF, as a prognostic biomarker may assist in the physician's decisions. In conclusion, clopidogrel, ticagrelor, prasugrel, and aspirin are all valuable drugs, but it is not easy to determine the strongest or identify if they can be substituted for one another. However, it is clear that genetic markers and coagulation factors can assist physicians' decision-making and increase the probability that a patient will have a safer future after anti-platelet therapy.

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Compliance with ethics guidelines

The authors declare no conflict of interest. This article does not contain any studies with human participants or animals that were performed by any of the authors.

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