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Review

A brief review on the mechanisms of aspirin resistance

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ABSTRACT

Aspirin is the most widely prescribed drug for the primary and secondary prevention of cardiovascular and cerebrovascular diseases. However, a large number of patients continue to experience thromboembolic events despite aspirin therapy, a phenomenon referred to as aspirin resistance or treatment failure. Aspirin resistance is often observed along with a high incidence of unstable plaque, cardiovascular events and cerebrovascular accident. Studies have shown that aspirin reduces the production of TXA2, but not totally inhibits the activation of platelets. In this review, we analyze current and past research on aspirin resistance, presenting important summaries of results regarding the potential contributive roles of single nucleotide polymorphisms, inflammation, metabolic syndrome and miRNAs. The aim of this article is to provide a brief review on aspirin resistance and platelet function, which will provide important insights into the research of aspirin resistance.

1. Introduction

Platelets are classically known as specialized effector that play a considerable role in the pathogenesis of vascular disease by adhering to ruptured unstable atherosclerotic plaques [1]. Recent studies have also shown that platelets are involved in immune and inflammatory responses [2,3]. Even though platelets are anuclear cell fragments, they have mRNA, miRNA and other molecules that regulate the post-transcriptional process through specialized pathways, which ultimately lead to variations in proteome, phenotype, and functions [4]. Aspirin is the most prescribed anti-platelet drug for primary prevention of cerebrovascular and cardiovascular disease in a broad range of population [5]. In addition, aspirin is also used for the secondary prevention of recurrent ischemic vascular events [6]. The major molecular target of aspirin is cyclooxygenase (COX). As an activator, COX converts arachidonic acid (AA) to thromboxane A2 (TXA2), a potent platelet agonist that displays prothrombotic properties. Aspirin modifies COX by acetylating COX-2 at serine 516 and COX1 at serine 529, spatially inhibiting AA binding and ultimately preventing AA transfer to TXA2 irreversibly [7,8]. In fact, the inhibition of platelet aggregation occurs at the megakaryocyte level even before the platelets are generated [9]. According to the Antiplatelet Trialists' Collaboration study, aspirin reduces the risk of myocardial infarction, stroke and death by approximately 25% compared to placebo [10]. Despite the efficacy of aspirin treatment on preventing cardiovascular and cerebrovascular diseases, aspirin does not have the same level of effect on all patients. In fact, about 5%–60% patients were reported poor antiplatelet effects after aspirin treatment, the phenomenon reported as "aspirin resistance", even if there is no consensual definition of aspirin resistance [11–13]. Generally, the Laboratory definition of aspirin resistance is the deficiency in inhibition of TXA2 or failing in reduction of platelet aggregation; which results in increase of TXA2 metabolites in urine and plasma. And the clinical aspirin resistance is used to be defined as failing in prevention of thrombus events in the patients [14]. This review aims to provide a brief mechanism summary on aspirin resistance, which include single nucleotide polymorphisms, inflammation, metabolic syndrome and miRNAs.

2. Single nucleotide polymorphisms and aspirin resistance

The surface of platelets contains lots of transmembrane Glucose Protein (GP) receptors, which are highly polymorphic and are encoded by two or more allelic isoforms. Single nucleotide polymorphisms (SNPs), which occur within a gene or in the regulatory regions near a gene, are the important contributors to residual platelet reactivity in patients with aspirin resistance [15]. The difference in amino acid sequences resulted from these SNPs might affect the secondary or tertiary structure of the GP receptors, and ultimately lead to corresponding changes in their biological function. Studies have identified SNPs that

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are significantly associated with the anti-platelet effect of aspirin and reported that hereditary factors account for about 30% of platelets' reactivity [16].

GP IIb/IIIa (also known as integrin αIIbβ3) is an integrin complex on the surface of platelets. Each of its subunits exists in different isoforms, which allows for considerable genetic variability. Genetic polymorphisms in GP IIIa are linked to differential response to aspirin therapy and are associated with an increased risk of thrombotic events [17]. The SNP rs5918 in the GP IIIa has been described as a thymidine to cytosine substitution in exon 2 of the GP IIIa gene, resulting in a leucine to proline mutation at amino acid 33 of the mature protein [18]. It has also been shown that the rs5918 C allele might play a role in platelet aggregation among healthy individuals, not just in patients with cardiovascular disease [19]. Individuals carrying SNP rs5918 also have higher risks of atherosclerotic plaque rupture [18]and ischemic stroke [20]. In addition, another study reported a potential correlation between rs5918C and myocardial infarction, although it was not significant (P = .083) [21].

GP Ia/IIa and GP VI are the important collagen receptors. GP Ia/IIa triggers the initial interaction of platelets and collagen, and platelets are subsequently activated by GPVI. Polymorphisms related to GP Ia/IIa and GP VI have been suggested to contribute to attenuation of the anti-platelet effect of aspirin [22]. The rs1126643 (C807T) of the GP Ia subunit, in particular, was suggested to have a prothrombotic effect regarding aspirin resistance in some studies [23–25]. However, a recent meta-analysis does not support a direct association between rs1126643 and either CAD or MI [26]. The SNP rs1613662 (T13254C) of GP VI leads to the substitution of serine 219 by proline and has been associated with coronary thrombus formation [27]. However, Voora et al. [28] found no clinical association between the SNPs of GP VI and thrombotic outcome in CAD patients. Sokol, J. et al. [29] has reported an association between rs1671153 and platelet aggregation in patients suffered from fetal loss. Peter Kubisz [30] has found that rs12610286 might be associated with platelet hyperaggregability in patients with sticky platelet syndrome, but other SNPs of the GP VI, such as rs1654410, rs1671153, rs1654419, rs11669150, rs1613662, and rs1654431, were not identify as frequently as rs12610286 in the same experimental group.

Von Willebrand factor (**vWF**) is a large multimeric glycoprotein produced by the endothelium and megakaryocytes. As a protective carrier molecule for clotting factor VIII, vWF mediates initial platelet adhesion at the site of vascular injury [31]. A meta-analysis study provided strong evidence that the SNP rs6065 of GP1bα is associated with ischemic stroke [32] Another SNP in vWF gene, rs1063856, has been shown to associate with the efficacy of tissue-type plasminogen activator in a Spanish population, and their mechanism of action might be associated with coagulation factor activity [33]. In addition, rs1063856 has also been reported to associate with an increased risk of vein thrombosis [34], but does not appear to associated with congestive heart failure [35].

COX is a key enzyme of the prostaglandins, thromboxane and prostacyclin pathway. There are two COX isozymes, constitutive COX-1 and inducible COX-2, which differ in their regulation of expression and tissue distribution. COX-1 is constitutively expressed in platelets, as well as gastric mucosa and kidneys. *In vivo* research has shown that inhibition of COX-1 could reduce the basal production of TXA2 in platelets, which might contribute to thrombosis. COX-2 is not expressed in endothelial cells and platelet under physiological conditions; however, elevated levels of COX-2 have been reported during oxidative stress and inflammation [36]. Genetic variability in COX-1 appears to regulate arachidonic acid-induced platelet aggregation and thromboxane generation. Therefore, heterogeneous response to aspirin might in part reflect variations in COX-1 genotype [37]. A study evaluated the impact of COX-1 gene polymorphisms on vascular events in a large cohort of Chinese patients with ischemic stroke after aspirin treatment, in which four alleles (rs1330344, rs10306114, rs3842788 and rs5788) were identified,but only rs1330344 was significantly associated with vascular events [38]. COX-2 is rarely expressed in most cells under normal conditions; however, elevated levels of COX-2 could be provoked by inflammation. Furthermore, polymorphisms of COX-2 have been shown to be in close relationships with diseases such as thrombosis and inflammation. A large-scale case–control study has demonstrated that rs20417 is associated with a significant reduction in the risk of cerebral and cardiac vascular events [38]. Other findings have provided evidence that rs20417 might increase the risk of aspirin resistance and clinical thrombus events [24,39].

P2Y1 and **P2Y12** belong to the family of G-protein coupled receptors and are found mainly on the surface of platelets. A study from mainland China showed that presence of the P2Y1 rs1065776 gene appears to attenuate the effect of aspirin during treatment in healthy volunteers [40]. Studies on the association between P2RY1 and P2RY12 polymorphisms and aspirin effect on platelet showed that P2RY12 SNP rs9859538 is associated with high residual platelet reactivity (RPR); P2RY12 SNPs, including rs1491974, rs3732765, rs10513398, and rs10935841, are moderately associated with RPR; rs7615865, rs1388623, rs1388622, rs7634096, and rs7637803 is slightly associated with RPR; P2RY1 SNPs, including rs1439010, rs701265, rs2312265, rs1371097 and rs12497578, are moderately associated with RPR [41]. In another study, nine P2Y12 gene polymorphisms have been described, among which four of them (rs10935838, rs2046934, rs5853517 and rs6809699) achieved complete linkage disequilibrium [42].

Thromboxane A2 Receptor is a protein that, in humans, is encoded by the TBXA2R gene, which exhibits a wide distribution in different cell types and different organ systems. SNPs in the TXA2 receptor gene affect the risk of developing cerebral ischemia and the function of platelet, in particular platelet aggregation [43]. A significant difference in the distribution of rs768963 between cerebral ischemia patients and control groups was reported in a Chinese population; however, no significant association was found between rs4523 variants and cerebral infarction [44]. The association between genetic polymorphisms and the anti-platelet effect of aspirin in 110 healthy Japanese individuals has been explored, which found that the 1018C and the 924T (rs4523) alleles are involved in aspirin resistance [45].

PharmGKB is a publicly accessible database that provides integrated knowledge including information on the associations of gene-drug and genotype-phenotype [46]. It is also a source of high-quality information important to personalized medicine projects. A set of 14 SNPs that have been suggested to associate with aspirin or thrombosis events selected from pharmGKB are illustrated in Table.1. However, level of evidence classification showed that only rs6065 and rs10306114 are moderately associated with aspirin resistance and that the other SNPs lack clear evidence of association. In summary, although pharmacogenetics studies provide molecular clues and clinical diagnostic tools on aspirin

Table 1
14 SNPs associated with aspirin or thrombosis events selected from pharmGKB.

Variant	Gene	Type	Level of evidence	Diseases
rs6065	GP1BA	Efficacy	2B	Aspirin resistance
rs10306114	PTGS1	Efficacy	2B	CAD, MI
rs20417	PTGS2	Efficacy	3	CAD
rs1126643	ITGA2	Efficacy	3	CAD, MI
rs1065776	P2RY1	Efficacy	3	MI
rs1613662	GP6	Efficacy	3	CAD
rs1062535	ITGA2	Efficacy	3	MI
rs2768759	NTRK1	Efficacy	3	Platelet aggregation
rs5985	F13 A1	Efficacy	3	Coagulation factor XIII activation
rs1062535	ITGA2	Efficacy	3	MI
rs10306114	PTGS1	Efficacy	3	MI
rs12041331	PEAR1	Toxicity/ADR	3	MI
rs5918	ITGB3	Efficacy	3	CAD, MI
rs4523	TBXA2R	Efficacy	3	CAD

Level of evidence: Level 2B: Annotation for a variant-drug combination with moderate evidence of association. Level 3: Annotation for a variant-drug combination based on a single significant, and lacking clear evidence of association.