The role of genetic factors in clopidogrel antiplatelet therapy

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Postep Kardiol Inter 2012; 8, 2 (28): 126–129
DOI: 10.5114/pwki.2012.29653

Abstract

Thienopyridine derivative antiplatelet agents play an important role in the treatment of coronary artery disease. Clopidogrel is a drug blocking action of the platelet ADP receptor ($P2Y_{12}$). It is applied in the form of a prodrug that requires metabolic activation by cytochrome P450 enzymes. The standard antiplatelet therapy in patients with acute coronary syndrome is based on simultaneous administration of clopidogrel and aspirin. Numerous scientific reports indicate that a diverse response to clopidogrel therapy is observed in as many as 25% of patients with acute coronary syndrome. This variation may be caused by genetic factors. Polymorphisms in CYP2C19 (one of the cytochrome P450 enzymes) and ABCB1 (gene coding P-glycoprotein which takes part in absorption of clopidogrel) are considered the most frequent genetic causes of resistance to clopidogrel therapy. Knowledge of these genotypes can be helpful in finding the patients vulnerable to less effective clopidogrel therapy and establishing an effective dose of the drug.

Key words: antiplatelet therapy, clopidogrel, genetic factors

Introduction

Coronary artery disease is one of the conditions depending on the type of atherosclerosis. The presence of atherosclerotic plaque leads to reduction of the coronary artery lumen and impaired coronary flow, restricting the transport of oxygen to a specified area of myocardial infarction. This results in ischemia and, in consequence, in chest pain. Clinical symptoms of coronary artery disease can manifest as chronic (stable angina) or acute (acute coronary syndrome, including unstable angina, myocardial infarction without ST-segment elevation or with ST-segment elevation and sudden cardiac death) [1]. A major role in the treatment of coronary artery disease is attributed, among others, to antiplatelet drugs, which are particularly important in the therapy of patients undergoing percutaneous coronary interventions, as the stents implanted in the coronary arteries are foreign objects to the body, causing platelet adhesion and activating prothrombotic processes [2]. Activation of platelets in the blood vessel affected by atherosclerosis is a process consisting of many individual steps, which have their origin in a transient interaction between the platelet and the endothelium. This interaction may become permanent, which ultimately leads to a cascade of events ending in thrombus formation and vessel occlusion [3]. If this situation occurs in the coronary arteries, it becomes the cause of myocardial infarction. Knowledge of various stages of platelet activation and additional factors influencing these processes enabled development of antiplatelet drugs using these mechanisms.

Mechanisms of platelet activation

Based on the current knowledge on the pathophysiology of blood vessels it was assumed that the initial contact between platelets and the intact endothelium is mediated through the von Willebrand factor or the endothelial surface protein P-selectin, both involved in the rolling of platelet at the endothelium. The von Willebrand factor also mediates the next stage of platelet activation, which is strong binding to the platelet GP Ib receptor resulting in the first strong adhesion. This initially reversible interaction may lead to platelet pre-activation through a release of factors mediating auto-activation, such as adenosine diphosphate (ADP) and thromboxane A2 (TxA_2) or factors with vasoactive properties, such as epinephrine, serotonin, and CD40 ligand (CD40L). The final stage of platelet activation takes place at the site of disrupted endothelium, when collagen fibers contact with flowing blood. This leads to a change of the platelet shape, increase of intracellular

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Praca wpłynęła: 27.04.2012, **przyjęta do druku:** 28.05.2012.

calcium levels and release of activating factors from the platelets. The platelet surface contains collagen receptors (GPIa/IIa, GPVI), which mediate their stable adhesion and strong activation. The aggregation cascade is sustained mainly by ADP and thromboxane, which activate inactive platelets. Platelet ADP receptors include $P2Y_1$ and $P2Y_{12}$, which is the target of thienopyridine drugs (such as clopidogrel) [3].

Clopidogrel characteristics

Clopidogrel is a thienopyridine derivative inhibiting the $P2Y_{12}$ receptor. Binding of the drug to the receptor prevents the connection of a physiological ligand (ADP) released from activated platelets [4, 5]. The functional forms of the $P2Y_{12}$ receptor are homo-oligomers. Clopidogrel causes the breakdown of these oligomers to inactive mono-and dimeric units. The study carried out by Savi *et al.* showed that the key element necessary for the activity of clopidogrel is a cysteine at position 97 of the $P2Y_{12}$ receptor protein chain [6]. Clopidogrel is administered as a prodrug, which requires metabolic activation by cytochrome P450 enzymes. Inhibition of ADP-induced platelet aggregation reaches a plateau after 5 days of standard therapy (75 mg daily). In the case vessel's serious injury (such as the one caused by stent implantation), the antiplatelet effect may be achieved after 2-5 h with administration of a loading dose (300-600 mg). Due to irreversible binding of clopidogrel to the $P2Y_{12}$ receptor, platelet function returns to normal about a week after the last dose of the drug. Clopidogrel is rapidly absorbed after administration, reaching peak plasma concentration (160 ng/ml) already during the first hour. The transport of clopidogrel involves glycoprotein P. Most of the drug (85%) is metabolized to an inactive carboxylate derivative. Only 15% is converted to the active metabolite in the two-step oxidation process conducted by cytochrome P450 (with 2-oxyclopidogrel as an intermediate form). The metabolism of clopidogrel is still not completely understood, but it is assumed that CYP3A4, CYP2C19 and CYP1A2 enzymes play a key role in this process [7]. Numerous studies, such as CAPRIE, CURE, CREDO, CLARITY and COMMIT, have shown that the use of combined antiplatelet therapy (aspirin and clopidogrel) significantly reduces the risk of cardiovascular events in patients suffering from cardiovascular diseases [8-12]. Based on these studies combined aspirin + clopidogrel therapy has become a standard of care for patients with acute coronary syndrome [4]. However, there are numerous reports indicating that up to 25% of patients with acute coronary syndrome undergoing percutaneous coronary intervention may present a variable response to clopidogrel. Patients resistant to the drug are exposed to recurrent cardiovascular events. The exact definition of resistance to antiplatelet therapy has not yet been determined, but the observed variation in response to therapy is similar to differences observed in patients treated with warfarin [13, 14].

The role of genetic factors in the varied response to clopidogrel

The varied response to clopidogrel may be associated with genetic factors. Genes whose polymorphisms may play a role in resistance to antiplatelet therapy can be predicted on the basis of pharmacokinetic and pharmacodynamic properties of the drug. Thus, clopidogrel is absorbed in the gastrointestinal tract with the participation of P-glycoprotein encoded by the ABCB1 gene. After absorption, it is inactivated by esterases or undergoes metabolic activation in the liver by cytochrome P450 enzymes (CYP1A2, CYP3A4, CYP3A5, CYP2C19). The activated drug binds irreversibly to the ADP receptor encoded by the P2RY12 gene, thereby inactivating the fibrinogen receptor, encoded by the ITGB3 gene. Numerous publications describe the impact of certain variants of these genes on the activity of clopidogrel.

Genes encoding cytochrome P450 enzymes seem to have the most important role, due to their high polymorphism and frequent occurrence of alleles encoding enzymes with reduced functionality (deficient alleles). Suh *et al.* studied the effect of CYP3A5 gene polymorphism on the efficacy of clopidogrel. The study was conducted in two stages. The first stage involved administration of clopidogrel to 16 healthy subjects who were carriers of deficient alleles in the CYP3A5 gene (allele *3) and to 16 healthy subjects with normal genotype (allele *1). Platelet aggregation was measured 4 h, 24 h and 6 days after the administration of clopidogrel. In the second stage of the study the clinical results of treatment with clopidogrel in 348 patients after coronary angioplasty were compared and associated with the CYP3A5 genotype. In the first stage there was a higher antiplatelet activity in patients with normal CYP3A5 genotype (*1). In the second stage the incidence of cardiovascular events during 6 months after stent implantation was higher in patients carrying the nonfunctional *3 allele (7.3% vs. 1.9%) [15].

The TRITON-TIMI 38 study examined the relationship between polymorphism of CYP2C19, CYP2C9, CYP2B6, CYP3A5, CYP3A4 and CYP1A2 genes and concentration of the active metabolite of clopidogrel together with the level of antiplatelet activity in 162 healthy subjects and 1477 patients with acute coronary syndrome. It was shown that healthy carriers of at least one deficient allele of the CYP2C19 gene (30% of the population) treated with clopidogrel had a 32.4% lower level of the active metabolite of clopidogrel in plasma in comparison to those with the normal genotype. Carriers of deficient alleles also showed a 9% lower antiplatelet activity of clopidogrel. In turn, patients with acute coronary syndrome who carried deficient alleles had a 53% higher risk of cardiovascular events and three times higher risk of in-stent thrombosis [16].

Lee *et al.* also confirmed the importance of CYP2C19 gene polymorphism. The study was carried out on 450 patients undergoing percutaneous coronary interventions and treated with standard combined therapy of aspirin +

clopidogrel (225 people) or with clopidogrel + aspirin + cilostazol (225 people). Polymorphism of the following 7 genes was analyzed: cyclooxygenase-2, CYP1A1, CYP1A2, CYP3A4, CYP3A5, CYP2C19*2 and CYP2C19*3. Resistance to clopidogrel was found in 112 patients. Patients resistant to clopidogrel had a significantly higher frequency of a deficient CYP2C19*3A allele, suggesting a role of this polymorphism as an independent risk factor [17].

The role of polymorphism in the CYP2C19 gene was also confirmed in the PAPI study. The study was carried out on a group of 429 healthy subjects who received 75 mg/d of clopidogrel for 7 days and subsequently underwent the assessment of treatment effect by means of *ex vivo* platelet aggregometry. Association studies found a significant relation between 13 single nucleotide polymorphisms (SNPs) located on chromosome 10 at the 10q24 locus and a reduced response to clopidogrel. One of these polymorphisms (rs12777823) was strongly associated with a deficient CYP2C19*2 allele and accounted for 12% of the variation in response to treatment. In the group of 227 patients after percutaneous coronary intervention who carried this polymorphism there was a 2-fold higher risk of cardiovascular events (20.9% compared to 10.0% in those with normal genotype) during 1-year follow-up [18].

Harmsze *et al.* examined polymorphism of 6 genes related to the absorption (ABCB1), activation (CYP2C9, CYP2C19, CYP3A4, CYP3A5) and function of clopidogrel ($P2Y_1$). The study included 428 patients after coronary stent implantation. Patients were divided into two groups: one receiving a maintenance dose of the drug (75 mg/day, ≥ 5 days before percutaneous coronary intervention, 297 patients) and the second receiving a loading dose of the drug (300 mg/day, 1-5 days before the percutaneous coronary intervention, 131 patients). The presence of the deficient CYP2C19*2 allele was significantly related to higher platelet activity and worse response to treatment in both groups. In addition, in the group of patients receiving a loading dose there was a highly significant relation between the presence of the CYP2C19*3 allele and reduced response to the drug [19].

Simon *et al.* also confirmed the role of genetic factors in the emergence of resistance to clopidogrel. The study group consisted of 2208 patients after acute myocardial infarction. The researchers assessed the relationship between allelic variants of genes involved in absorption (ABCB1), metabolic activation (CYP3A5, CYP2C19), and biological activity of clopidogrel (P2RY12, ITGB3) and the risk of death, stroke or myocardial infarction during 1-year observation. During the study 225 patients died and 94 suffered from stroke or myocardial infarction. A higher incidence of cardiovascular events (15.5% vs. 10.7%) was found in patients who carried the deficient allele of the ABCB1 gene (TT homozygotes at nucleotide 3435) in comparison to individuals carrying a normally functioning allele (CC homozygotes). In addition, patients who carried any two deficient alleles of CYP2C19 (*2, *3, *4, *5) had a higher incidence of cardiovascular events in comparison to patients with normal genotype (21.5% and 13.3%). Moreover, the incidence of cardiovascular events among the 1535 patients who underwent PCI was 3.58 times higher in patients who carried two CYP2C19 deficient alleles [20].

There were also studies comparing the influence of genetic factors on treatment with different thienopyridine derivatives. Varenhorst *et al.* studied 98 patients with coronary artery disease treated with clopidogrel (loading dose 600 mg/maintenance dose 75 mg) or prasugrel (60 mg/10 mg). The analyzed genes included CYP2C19, CYP2B6, CYP2C9, CYP3A5, CYP3A4, and CYP1A2. Patients were divided into two groups based on the genotypes: fast metabolizers (with normally functioning enzymes), and slow metabolizers (with reduced functionality of the enzymes). A significantly lower level of the active metabolite of clopidogrel was found in patients carrying the deficient CYP2C19 gene allele as compared to patients with normal genotype. Such relations were not detected for prasugrel [21].

Interesting conclusions about the role of CYP1A2 and the activity of clopidogrel result from the work of Desai *et al.* (2009; the CLARITY-TIMI study). CYP1A2 is one of the key enzymes that metabolize clopidogrel to its active form. This enzyme is also induced during cigarette smoking. A group of 3429 patients after ST-segment elevation myocardial infarction was divided into a non-smoking subgroup (1732 patients) and subgroups smoking 1-9 cigarettes per day (206 patients), 10-19 cigarettes per day (354 patients), 20-29 cigarettes per day (715 patients) and over 30 cigarettes per day (422 patients). The efficacy of clopidogrel was higher in those smoking at least 10 cigarettes per day (odds ratio 0.49, 95% confidence interval CI 0.37-0.66, $p < 0.0001$) in comparison to non-smokers (odds ratio 0.72, 95% confidence interval CI 0.57-0.91, $p = 0.006$) [22].

Conclusions

Genetic factors significantly influence functioning of many drugs, including clopidogrel. Numerous studies conducted so far indicate that ABCB1 and CYP2C19 polymorphisms appear to have the greatest importance. Determination of genotype for these two loci in patients treated with clopidogrel may be helpful in the identification of patients exposed to lower efficacy of the drug and in the selection of an effective dose, thus improving the efficacy and safety of the therapy.

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