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Review Article

Pharmacogenomics of clopidogrel: Evidence and perspectives

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ABSTRACT

Clopidogrel has become the mainstay oral antiplatelet regimen to prevent recurrent ischemic events after acute coronary syndromes or stent placement. However, there is marked interindividual variability in the antiplatelet effects of clopidogrel, and a reduced response to this drug may be a risk factor for ischemic complications. Pharmacogenomic analyses, including candidate-gene and genome-wide association studies, have confirmed that genetic polymorphisms in the hepatic cytochrome P450 (CYP) 2C19 dominantly affect the antiplatelet effects of clopidogrel. *CYP2C19* reduced-function alleles have been associated with a significant decrease in clopidogrel responsiveness and a higher risk of adverse cardiac events including stent thrombosis, myocardial infarction, and death in several prospective studies, although these effects were not reproduced in a recent large randomized study that included a randomized control group. The US Food and Drug Administration addressed this issue by adding a boxed warning to the clopidogrel label and suggesting that adjusting the clopidogrel dose or using alternative antiplatelet agents should be potentially implemented for high-risk individuals who are identified based on the *CYP2C19* genotype. Although it is promising that *CYP2C19* genotyping could be used to guide personalized antiplatelet clopidogrel therapy, currently there is insufficient evidence to recommend routine genetic testing. Prospective randomized clinical trials are necessary to validate this pharmacogenomic approach to clopidogrel therapy. In the most recent trial, paraoxonase-1 (PON1) was identified as a crucial new enzyme for clopidogrel bioactivation, with its common Q192R polymorphism determining the rate of active metabolite and the clinical activity of clopidogrel. Further studies are needed to investigate the comprehensive influence of a number of different polymorphisms of *CYP2C19* and *PON1* variant alleles or other genetic variants on clopidogrel in various ethnic populations.

Contents

Abbreviations: CYP, cytochrome P450; paraoxonase-1, PON1; PCI, percutaneous coronary intervention; ADP, adenosine diphosphate; cAMP, cyclic adenosine monophosphate; VASP, vasodilator-stimulated phosphoprotein; OR, odds ratio; CI, confidence interval; PPI, proton-pump inhibitors.

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Introduction

The antiplatelet drug clopidogrel is an important therapeutic agent that is used on top of aspirin to provide additive efficacy and overcome aspirin resistance in patients who are at risk for cardiovascular events [1]. Clopidogrel plus aspirin is recommended in the American College of Cardiology/American Heart Association guidelines and has become the standard of care for patients with acute coronary syndrome [2–5]. In combination with aspirin, clopidogrel is also the gold standard to prevent stent thrombosis in patients undergoing percutaneous coronary intervention (PCI) [2,6]. However, the antiplatelet effects of clopidogrel are not consistent in all patients [7–9]. Despite adequate antiplatelet therapy, up to 15% of high-risk patients with acute coronary syndrome continue to suffer from ischemic events, and up to 30% of the patient populations have marked interindividual variability in the extent of platelet inhibition [10]. Individual differences in the rate of platelet activation and reactivity markedly influence normal hemostasis and the pathological outcome of thrombosis. Clopidogrel resistance has been recognized in patients that exhibited less platelet aggregatory inhibition, and the prevalence of this resistance varied widely from 4% to 30%, with the value depending in part on the loading dose of clopidogrel and the methods used to assess non-responsiveness [11,12]. The inter-patient variability in the response to clopidogrel is multi-factorial and largely influenced by environmental, clinical and genetic factors. Among the environmental and clinical factors, age, smoking, diet, drug—drug interactions, diabetes, acute coronary syndrome, patient compliance, triglycerides, high-density lipoprotein cholesterol and body mass index may cause variability in the response to clopidogrel [13–16].

Clopidogrel is a prodrug and therefore must be metabolized before it can inhibit adenosine diphosphate (ADP)-induced platelet aggregation. The conversion of clopidogrel to its active metabolite is catalyzed by several different cytochromes P450s (CYPs) and an esterase paraoxonase-1 (PON1). Recent data demonstrated that individuals with *CYP2C19* and *PON1* gene variants have low active metabolite levels of clopidogrel [17–20]. Increasing evidence suggests that *CYP2C19* variants greatly influence the marked interindividual heterogeneity of the clopidogrel response and are linked to an increased risk of adverse cardiovascular outcomes, including stent thrombosis, myocardial infarction, and death [13,19,21–26]. Data are limited for the influence of *PON1* genotypes on the pharmacokinetics and pharmacodynamics of clopidogrel. Based on the vital pharmacogenomic information, personalized clopidogrel antiplatelet therapy is becoming a promising treatment approach.

In this review, we focus mainly on outlining the genetic polymorphisms associated with the variability in antiplatelet response to clopidogrel and the difference in clinical outcomes among these genotypes. Additionally, we review the perspectives on pharmacogenomic findings that relate to personalized antiplatelet clopidogrel therapy in the clinical setting.

Search methodology

We searched PUBMED and MEDLINE of the English-language literature from January 1989 to January 2011 using the following search items in different combinations: “clopidogrel,” “thienopyridine,” “resistance,” “platelet responsiveness,” “genetic polymorphism,” “pharmacogenomics,” “pharmacogenetics,” “clinical outcome” and “primary endpoints.” After reviewing the abstracts, we obtained and reviewed the full text of the relevant articles and their reference lists. Additional citations were obtained from the articles retrieved from the literature search.

Antiplatelet mechanism and metabolism of clopidogrel

Platelets have a central role in the development of atherothrombotic events, and therefore antiplatelet therapy has become the cornerstone for cardiovascular disease management [27]. Clopidogrel is used to treat arterial thrombosis because this drug pharmacologically targets the platelet receptor systems. The key mediator of platelet activation and aggregation is ADP, which binds and activates platelets through two G-protein coupled receptors, $P2Y_1$ and $P2Y_{12}$ (Fig. 1). The $P2Y_{12}$ receptor is coupled to Gi and is responsible for platelet aggregation, especially its stabilization. The $P2Y_{12}$ receptor amplifies cytoplasmic Ca^{2+} mobilization that is mediated by the $P2Y_1$ receptor and induces the secretion of dense granules via stimulation of secretion-inducing agonists such as thromboxane A2 and thrombin. Several studies have suggested that phosphatidylinositol 3-kinase plays an important role in ADP-dependent, $P2Y_{12}$ receptor-mediated potentiation of platelet activation [28]. However, the signal transduction pathways that are mediated through the $P2Y_{12}$ receptor are not well understood. Since clopidogrel reduces the cyclic adenosine monophosphate (cAMP) levels by inhibiting adenylate cyclase by Gi, the effectiveness of clopidogrel can be directly monitored based on flow cytometric analyses of the phosphorylated vasodilator-stimulated phosphoprotein (VASP-P) levels [29].

Clopidogrel is a prodrug that must be converted into an active thiol-containing metabolite before it can express antiplatelet function [30]. The active metabolite covalently binds to the platelet $P2Y_{12}$ receptor and irreversibly inhibits ADP-stimulated platelet aggregation [30–32]. Pharmacokinetic studies indicate that clopidogrel is converted into its active metabolite by hepatic CYPs in a two-step oxidation process (Fig. 1). The first oxidative step is catalyzed by CYP2C19, CYP1A2 and CYP2B6, where each enzyme is responsible for 45%, 36% and 19% of the conversion, respectively. The second step is mediated by CYP3A4, CYP2B6, CYP2C19 and CYP2C9, where each enzyme is responsible for 40%, 33%, 21% and 7% of the conversion, respectively [33]. Thus, CYP2C19 substantially contributes to both oxidative steps that generate the active clopidogrel metabolite. However, in a recent study using recombinant metabolizing enzymes in a microsomal fraction expressed in HEK293 cells, Bouman *et al.* found that CYP3A4, CYP3A5, CYP2B6, and CYP1A2 were the first oxidative step enzymes, and PON1, an esterase synthesized in the liver and associated with high density lipoprotein in the blood, emerged as the rate-limiting enzyme for the second step of hydrolytic cleavage of the γ-thiobutyrolactone ring of 2-oxo-clopidogrel [20]. Approximately 15% of clopidogrel is available as an active metabolite and the remaining 85% is hydrolyzed to an inactive carboxylic acid derivative compound by esterases [34].

Genetic polymorphisms in *CYP2C19* that are relevant to clopidogrel

Deleterious genetic variants of CYP2C19

Although the active metabolite of clopidogrel arises from complex biochemical reactions that involve a number of different hepatic CYP enzymes, there is accumulating evidence that CYP2C19 plays a dominant role in clopidogrel activation [19,33,35]. The *CYP2C19* gene is located on chromosome 10 (10q24.1-q24.3) and consists of 490 amino acid residues. *CYP2C19* is one of the most polymorphic CYP genes among diverse racial groups. At least 25 genetic variants in *CYP2C19* have been identified [36]. Among these, two loss-of-function variant alleles, *CYP2C19*2* and **3*, account for the majority of the defective genotypes. *CYP2C19*2* (rs4244285) carries a 681G>A change in exon 5, which produces an aberrant splice site and leads to a truncated nonfunctional protein [37]. *CYP2C19*3* (rs4986893) has a 636G>A change in exon 4 that produces a premature stop codon [38]. Recently, a mutation in the regulatory region, *CYP2C19*17*, characterized by a -806C>T change in the 5’-flanking region of the gene was reported to be associated with increased CYP2C19 expression and enzymatic activity [39]. Additional missense mutations in *CYP2C19* that are relevant to defective enzyme function are summarized in