A TECHNICAL REVIEW

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Clopidogrel *CYP2C19* Genotyping

Introduction

Clopidogrel bisulfate (Plavix®) is an antiplatelet agent used in the treatment of individuals with cardiovascular atherosclerotic disease. Patients with myocardial infarction, stroke, transient ischemic attacks, or unstable angina managed medically or with percutaneous intervention are all candidates for therapy with clopidogrel.[1] Despite the availability of dual platelet inhibitor treatment (clopidogrel plus aspirin) for unstable coronary syndromes, the American Heart Association estimates that there will be 470,000 recurrent cardiac events in 2009.[2]

Significant interindividual variability has been observed in the therapeutic response to clopidogrel, with some patients exhibiting "resistance" to antiplatelet therapy.[3] Poor pharmacodynamic response to clopidogrel is associated with a higher risk of arterial thrombotic events.[4] Many factors, including age, compliance with therapy, clinical status, as well as genetics may play a role in variable response to clopidogrel therapy.

As investigators have searched for genetic modulators of clopidogrel response, several studies have identified genetic variation in the cytochrome P450 *2C19* gene, required for oxidative activation of clopidogrel, as a key genetic factor associated with clinical response to clopidogrel therapy.[5-9] Evidence of decreased effectiveness of clopidogrel in *CYP2C19* poor metabolizers has led the US Food and Drug Administration (FDA) to add a boxed warning to the drug label to inform physicians about the increased risk of cardiovascular events in poor metabolizers.[1] The boxed warning also suggests that alternate treatment or treatment strategies be considered in patients identified as poor metabolizers.[1]

Clopidogrel Mode of Action

Clopidogrel is a prodrug that requires biotransformation by hepatic cytochrome P450 (CYP) enzymes into an active metabolite. Following intestinal absorption, most of the prodrug is metabolized into inactive metabolites by ubiquitous esterases. A minor fraction of the prodrug is oxidized in the liver to its active form by the *2C19* metobolizing enzyme.

Once activated, clopidogrel exerts its effect by irreversibly binding to a specific adenosine diphosphate (ADP) platelet receptor (P2RY12), preventing ADP binding and subsequent activation of the fibrinogen receptor (glycoprotein [GP] IIb/IIIa), thus inhibiting platelet aggregation.[1] Clopidogrel also blocks the amplification of platelet response from agonists other than ADP by its effect on the platelet receptor.[1]

Cytochrome P450 (CYP) 2C19

Cytochrome P450 is a family of genes that encodes various drug-metabolizing enzymes that function primarily in the liver. The gene *2C19* codes for an enzyme involved in the metabolism of clopidogrel as well as several other drugs, including proton pump inhibitors (omeprazole), anticonvulsants (phenytoin and diazepam), and tricyclic antidepressants (amitriptyline and nortriptyline).

Loss of enzyme metabolizing function can be caused by single nucleotide polymorphisms in the gene that encodes the *CYP2C19* enzyme. Individuals carrying two wild-type (normal) alleles are considered extensive metabolizers (EMs). Those with one loss of function allele are intermediate metabolizers (IMs), and those with two loss-of-function alleles are poor metabolizers (PMs). The vast majority of *CYP2C19* poor metabolizers are accounted for by two common loss-of-function alleles *CYP2C19**2 (681G>A) and *CYP2C19**3 (636G>A). Other less common alleles associated with absent or reduced metabolism include *4, *5, *6, *7, and *8.[10] Loss-of-function alleles are not uncommon in the general population. Poor metabolizers (individuals with two loss-of-function alleles) occur at a frequency of 1% to 6% in Caucasians, 1% to 7.5% in African American populations, and 12% to 23% in Asian populations.[10]

Beyond the poor metabolizer alleles mentioned above, a recently identified *CYP2C19* allelic variant (*17) (– 806C>T) has been associated with increased transcription of the *CYP2C19* gene and with ultrarapid metabolism of *CYP2C19* substrates.[9,11] *CYP2C19**17 carriers appear to have an enhanced response to clopidogrel and may be at increased risk for bleeding.[9]

Cytochrome P450 2C19 Variants and Clopidogrel Response

Several studies have investigated the association between *CYP2C19* genotype and clinical outcomes in patients receiving clopidogrel therapy. In a study of 1477 clopidogrel-treated subjects with acute coronary syndromes carriers of at least one *CYP2C19* loss-of-function allele had a higher rate of major adverse cardiovascular events such as myocardial infarction, or stroke compared with noncarriers (12.1% vs 8% P = 0.01). There was also an increase in the risk of stent thrombosis by a factor of 3 (2.6% vs 0.8%; hazard ratio, 3.09; 95% CI, 1.19-8.0; P = 0.02).[6]

In another recent study of 2208 patients presenting with acute myocardial infarction, patients carrying two *CYP2C19* loss-of-function alleles had a higher event rate than patients without reduced function alleles (21% vs 13.3%; hazard ratio, 1.98; 95% CI, 1.10–3.58; P = 0.003).[5] In a subset analysis of patients who underwent percutaneous coronary intervention during hospitalization (n = 1535), the rate of cardiovascular events among patients with two *CYP2C19* loss-of-function alleles was 3.58 times the rate observed in those with none (95% CI 1.71–7.51, P = 0.005).[5]

There is a strong physiologic basis for the association between poor metabolizer *CYP2C19* alleles and an increased risk of cardiovascular events. Studies have shown that *CYP2C19* loss-of-function alleles

result in lower plasma exposure to the active metabolite.[6,12] Mega et al showed that carriers of at least one *CYP2C19* reduced function allele had a 32.4% reduction in circulating active clopidogrel metabolite compared with noncarriers ($P < 0.001$).[6]

Platelet aggregation measurements have also documented a decrease in the antiplatelet effect of clopidogrel among carriers of reduced function *2C19* alleles.[8,13] Patients with reduced antiplatelet response to clopidogrel appear to be at increased risk for recurrent cardiovascular events.[8,14,15] Ultrarapid *CYP2C19* metabolizers (*17 carriers), on the other hand, appear to have increased exposure to the active metabolite as evidenced by lower ADP-induced platelet aggregation and an increased risk of bleeding.[9] The clinical impact of the combination of an ultrarapid metabolizer allele with a poor metabolizer allele is not yet known and awaits further studies.

Monitoring Clopidogrel Therapy

Clopidogrel effect can be evaluated by measuring ADP-induced platelet aggregation or by analysis of P2RY12-mediated phosphorylation by flow cytometry.[16] Platelet function testing has the advantage of measuring the culmination of clopidogrel effect, which takes into account a host of effects and relationships, such as baseline platelet activity, drug metabolism, and platelet receptor activity.

Platelet function testing, however, is not readily available in many areas of the US. This is in part because testing is complex and must be performed within one to two hours of specimen collection. Furthermore, platelet function testing is not standardized, and clear definitions of appropriate clopidogrel response are lacking.[4] Without monitoring antiplatelet effect or determination of *CYP2C19* variants associated with reduced clopidogrel effect, identification of clopidogrel resistance relies on clinical occurrence of a recurrent atherothrombotic event.

Future Research

Metabolism by *CYP2C19* enzymes, and activation of clopidogrel, can be inhibited by coadministration of potent *CYP2C19* inhibitors.[17,18] The concentration of the active metabolite of clopidogrel was decreased, and inhibition of platelet aggregation diminished, when Plavix and omeprazole (Prilosec®), a proton pump inhibitor used to reduce stomach acid, were administered together.[19] As a result, on November 17, 2009, the FDA issued new safety information regarding the concomitant use of *CYP2C19* inhibitors, (eg, omeprazole).[20] The magnitude of the effect remains controversial, however, and additional studies are needed to clarify its impact.[21,22]

Several important questions remain regarding the clinical management of individuals who are identified as poor metabolizers by *CYP2C19* genetic testing and who are not able to metabolize clopidogrel fully to its active metabolite. Current trials are in progress to investigate treatment strategies for patients with *CYP2C19* polymorphisms that affect drug metabolism. Can reduced activation of the drug clopidogrel, as defined by a *CYP2C19* genotype, be safely overcome by increasing the dose of clopidogrel? Would individuals who carry *CYP2C19* loss-of-function alleles have improved outcomes if they were prescribed an alternative antiplatelet therapy (eg, prasugrel) that does not require activation by a *CYP2C19* enzyme? Ongoing prospective studies should provide answers to some of these questions and additional guidance for individualized therapeutic decisions and better health outcomes.

Clopidogrel CYP2C19 Genotyping 511710

For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at **www.LabCorp.com.**

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