Pharmacogenomics of antiplatelet drugs

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Clopidogrel, a platelet $P2Y_{12}$ inhibitor, is one of the most widely prescribed drugs in cardiovascular medicine because it reduces ischemic and thrombotic complications. It is a prodrug requiring biotransformation into the active metabolite by the hepatic cytochrome 450 system, especially the CYP2C19 enzyme. Candidate gene studies and genome-wide association studies have identified loss-of-function *CYP2C19* variants to be associated with a diminished pharmacologic response. Specifically, compared with noncarriers, carriers of at least one copy of a loss-of-function *CYP2C19* allele have ~30% lower levels of active clopidogrel metabolite and ~25% relatively less platelet inhibition with clopidogrel. Moreover, in patients treated with clopidogrel predominantly for percutaneous coronary intervention, carriers of 1 or 2 *CYP2C19* loss-of-function alleles are at increased risk for major adverse cardiovascular outcomes, with an ~1.5-fold increase in the risk of cardiovascular death, myocardial infarction, or stroke as well as an ~3-fold increase in risk for stent thrombosis. Tripling the dose of clopidogrel in carriers of a *CYP2C19* loss-of-function allele can achieve on-treatment platelet reactivity comparable to that seen with the standard 75 mg dose in wild-type individuals, but the impact on clinical outcomes remains unknown. Alternatively, 2 third-generation $P2Y_{12}$ inhibitors are available: prasugrel and ticagrelor. These drugs are superior to clopidogrel in reducing ischemic outcomes and are unaffected by *CYP2C19* loss-of-function alleles.

Learning Objective

- To understand the importance of pharmacogenetics with regard to the pharmacologic and clinical efficacy of $P2Y_{12}$ ADP receptor inhibitors

Background

Clopidogrel, an inhibitor of the $P2Y_{12}$ ADP receptor on the surface of platelets, is one of the most widely prescribed drugs in cardiovascular medicine. Clopidogrel, or earlier generations of $P2Y_{12}$ inhibitors, have been shown to reduce the risk of adverse clinical outcomes in patients with coronary disease in 2 major settings. First, among patients undergoing coronary stenting, dual antiplatelet therapy with aspirin and a $P2Y_{12}$ ADP receptor inhibitor reduces the risk of death and ischemic complications, including stent thrombosis, by 75%–85% compared with aspirin monotherapy or aspirin plus an anticoagulant.[1,2] Second, among patients presenting with an acute coronary syndrome (ACS), the addition of clopidogrel to aspirin reduces the risk of death and ischemic complications by ~20%.[3-5]

Clopidogrel, a thienopyridine, is a prodrug. Absorption of clopidogrel is limited by the intestinal efflux transporter P-glycoprotein.[6] Upon absorption, 85% of the prodrug is hydrolyzed by esterases into an inactive carboxylic acid derivative. The remaining 15% of the prodrug is metabolized by the hepatic cytochrome 450 system, especially the CYP2C19 enzyme, into an active thiol metabolite. It has been postulated that paraoxonase also plays a role in the biotransformation of clopidogrel,[7] although subsequent studies have questioned this supposition.[8-10]

After a typical 300 or 600 mg loading dose of clopidogrel, peak plasma concentrations of the active metabolites are reached within several hours.[11] The active thiol metabolite irreversibly binds the $P2Y_{12}$ ADP receptor on the platelet surface, thereby inhibiting ADP-dependent platelet activation and aggregation. Given that the typical lifespan of a platelet is 7-10 days, a return to normal platelet reactivity in an individual occurs over several days.

Variable response to clopidogrel

Variable platelet inhibition with clopidogrel therapy has been observed and approximates a bell-shaped distribution, with close to 1/3 of subjects not having an appreciable decrease in platelet reactivity after a standard 300 mg loading dose. Several studies have gone on to show that patients treated with clopidogrel who have high on-treatment platelet reactivity have a higher rate of ischemic complications such as myocardial infarction and stent thrombosis and, conversely, as would be expected, lower rates of bleeding.[12,13]

Drug–drug interactions have been investigated as potential contributors to the variable response to clopidogrel. In particular, some proton pump inhibitors (PPIs), such as omeprazole, are both inhibitors of and substrates for the CYP2C19 enzyme, which is important for clopidogrel metabolism. Initial observational data raised concerns about potential association of concurrent PPI (especially omeprazole) and clopidogrel therapy with increased cardiovascular events and mortality.[14] Several carefully done pharmacokinetic and pharmacodynamic studies have demonstrated that concomitant use of omeprazole can decrease levels of the active clopidogrel metabolite by 40%–45% and decrease platelet inhibition by ~20%.[15] In contrast, retrospective subgroup analyses of the TRITON-TIMI 38 and the FAST-MI registry found no association between concurrent PPI therapy and increased cardiovascular events and mortality.[16,17] Moreover, the prospective COGENT trial that randomized patients to clopidogrel with or without concomitant omeprazole reported no difference in the primary cardiovascular safety end point (defined as the composite of death from cardiovascular causes, myocardial infarction, coronary revascularization, or

ischemic stroke).[18] However, it should be noted that clopidogrel would not be expected to alter the likelihood of elective coronary revascularization and that there were relatively few events of the type that clopidogrel would be expected to affect (death from cardiovascular causes, myocardial infarction, or ischemic stroke). Therefore, the U.S. Food and Drug Administration (FDA) continues to mandate that the clopidogrel prescribing information include the following note: "CYP2C19 inhibitors: Avoid concomitant use of omeprazole or esomeprazole." Given the benefit in reduction of gastrointestinal bleeding in patients concomitantly taking PPIs with clopidogrel,[18] a 2010 expert consensus document recommended that "PPIs are appropriate in patients with multiple risk factors for GI bleeding who require antiplatelet therapy. Routine use of either a PPI or an H2RA (H2-receptor antagonist) is not recommended for patients at lower risk of upper GI bleeding."[19] Given that PPIs are available that do not inhibit CYP2C19 as strongly (eg, pantoprazole) and do not appear to influence platelet inhibition with clopidogrel,[15] it would be prudent to choose such a drug over PPIs that are known CYP2C19 inhibitors.

Pharmacogenetics

Polymorphisms of genes involved in the absorption (*ABCB1*) and metabolism (*CYP2C19*, possibly *PON1*) of clopidogrel have been investigated for potential association with clopidogrel response.

CYP2C19

The *CYP2C19* gene encodes for the cytochrome 450 2C19 enzyme, which is involved in both steps of hepatic activation of clopidogrel to its active metabolite. The *CYP2C19* gene is polymorphic, with known loss-of-function and gain-of-function variants. Among the loss-of-function variants, such as the *2, *3, *4, *5, *6, *7, *8 variants per the Karolinska Institute nomenclature, the *2 variant is the most common, with nearly 30% of a Caucasian population carrying 1 or 2 copies. The *2 variant (rs4244285) involves a single base pair mutation of G → A at position 681, which creates an aberrant splice site, resulting in downstream synthesis of a truncated nonfunctional CYP2C19 protein.

Several candidate gene studies and a genome-wide association study have identified loss-of-function *CYP2C19* variants to be independently associated with diminished inhibition of ADP-induced platelet aggregation.[20-24] Specifically, compared with noncarriers, carriers of at least one copy of a loss-of-function *CYP2C19* allele have ~30% lower levels of active clopidogrel metabolite and ~25% relative less platelet inhibition with clopidogrel.[23] In a cohort of patients treated with clopidogrel predominantly for percutaneous coronary intervention (PCI), carriers of both 1 and 2 *CYP2C19* loss-of-function alleles were at increased risk for major adverse cardiovascular outcomes, with a 1.5-fold increase in the risk of cardiovascular death, MI, or stroke and a 3-fold increase in risk for stent thrombosis compared with noncarriers.[23] This association was confirmed in a meta-analysis of 9 studies of close to 10 000 patients (>90% of whom underwent PCI).[22-28]

In contrast, genetic association studies of patients receiving clopidogrel who were predominantly medically managed (ie, did not undergo PCI) did not show an association between *CYP2C19* loss-of-function variants and major adverse outcomes.[29,30] Similarly, and not surprisingly, meta-analyses that have included studies in which the patient population has been shown not to benefit from clopidogrel, the outcomes were ones that clopidogrel has been shown not to alter, or patients were followed well beyond their last dose of clopidogrel have also not shown a significant association.[31] The strength of a pharmacogenetic interaction will naturally depend on the efficacy of the pharmacologic agent.[32] As noted earlier, in patients undergoing coronary stenting, dual antiplatelet therapy with aspirin and a $P2Y_{12}$ ADP receptor inhibitor reduces the risk of death and ischemic complications by 75%–85% compared with aspirin monotherapy or aspirin plus an anticoagulant.[1,2] Therefore, if a genetic variant were to completely inhibit the biotransformation of clopidogrel, then the subgroup with that variant should have an event rate similar to that in the aspirin monotherapy arm in the above trials. Therefore, the risk ratio in that subgroup versus wild-type patients would be the inverse of the benefit of $P2Y_{12}$ inhibition in that population or 1/0.20 = 5.0. Due to redundancy in the system, the presence of the *CYP2C19**2 allele does not completely prevent the biotransformation of clopidogrel, but reduces levels of the active metabolite by ~33% and of platelet inhibition by ~25%. For the sake of argument, if one were to assume a linear relationship between platelet inhibition and reduction in major adverse clinical events, then one would estimate that the presence of the *CYP2C19* *2 allele would confer a risk of ~30% of 5.0 (on a log scale) or ~1.6, which is quite similar to what has been observed. In contrast, for patients who are predominantly medically managed, the addition of clopidogrel to aspirin reduces the risk of death and ischemic complications by ~20%.[3-5] Doing the same calculations, complete genetic blockade would result in a risk ratio of 1/0.80 = 1.25. Partial genetic blockade would then be expected to result in a risk ratio of only 1.07, which would be extremely hard to detect and of only modest clinical importance.

The enhanced function *CYP2C19**17 variant has also been reported to influence the response to clopidogrel. The *CYP2C19**17 variant involves a single base pair mutation of C → T at position 808. The *CYP2C19**17 variant has been associated with increased transcriptional activity of the CYP2C19 enzyme, more extensive clopidogrel metabolism with enhanced production of active clopidogrel metabolites, and greater inhibition of ADP-induced platelet aggregation. Clinically, it has been associated with an increased risk of bleeding in a gene-dose-dependent fashion without significant impact on stent thrombosis or the combined 30-day ischemic end point of death, myocardial infarction, or urgent target vessel revascularization.[33] Genetic analysis of the CURE study found carrier status of the *CYP2C19*17* allele to be associated with more pronounced reduction of cardiovascular events with clopidogrel therapy, but no difference in bleeding.[29]

ABCB1

The *ABCB1* gene (also known as *MDR1*) encodes for the xenobiotic efflux p-glycoprotein pump involved in intestinal absorption of clopidogrel. The C3435T polymorphism has been variably associated with clopidogrel response. The 3435TT genotype has been associated with decreased peak plasma concentrations of clopidogrel and its active metabolites.[6] In the FAST-MI registry, patients who were 3435TT homozygotes versus CT/CC individuals had an ~70% increase in cardiovascular events in the setting of treatment with clopidogrel therapy after an acute myocardial infarction.[35] Likewise, in the setting of treatment with clopidogrel in TRITON-TIMI 38, *ABCB1* 3435 TT homozygotes experienced a 72% increased risk of adverse cardiovascular events compared with CT/CC individuals.[34] Data from the PLATO trial, though, provided contrasting results, with an association between the 3435CC genotype and higher rates of ischemic events.[35]

PON1

The *PON1* gene encodes for paraoxonase-1, an esterase synthesized in the liver and associated with HDL in the blood. Using *in vitro* metabolomic profiling, a team of investigators reported data suggesting that paraoxonase-1 played an important role in the bioactivation of clopidogrel from the intermediate product formed by the cytochromes in the liver to the active metabolite in the bloodstream.[7] The same team then performed clinical studies in which the *PON1* Q192R polymorphism was associated with the risk of ischemic events in patients treated with clopidogrel.[7] However, it should be noted that the *PON1* Q192R was not associated with clopidogrel pharmacologic effect in the previously published genome-wide association study of clopidogrel pharmacogenomics,[24] and that the very study that identified this novel *PON1* Q192R polymorphism was unable to reproduce the well-replicated effect of *CYP2C19* loss-of-function alleles on clopidogrel therapy. Subsequent pharmacology studies have now questioned the supposition that paraoxonase-1 plays a role in the bioactivation of clopidogrel.[8-10] Moreover, subsequent clinical studies have not supported a role for the Q192R polymorphism affecting cardiovascular outcomes in patients treated with clopidogrel.[36-46] Therefore, the weight of the evidence does not support genetic variants in PON1 affecting clinical outcomes in patients treated with clopidogrel.

Therapeutic implications

In March 2010, the FDA approved a new label for Plavix, with the addition of a boxed warning on pharmacogenetics, noting diminished effectiveness of therapy in poor metabolizers (defined as having 2 loss-of-function *CYP2C19* alleles). The boxed warning further states that "tests are available to identify a patient's *CYP2C19* genotype and can be used as an aid in determining therapeutic safety [and to] consider alternative treatment or treatment strategies in patients identified as *CYP2C19* poor metabolizers."[47] A variety of genotyping tests, including several point-of-care tests, have been developed and are now available.[48,49]

Escalating doses of clopidogrel

Potential therapeutic modifications for individuals found to carry a loss-of-function *CYP2C19* allele include escalation of clopidogrel dosage or switching to an alternate agent. The ELEVATE-TIMI 56 trial demonstrated that tripling the maintenance dose of clopidogrel to 225 mg daily in *CYP2C19**2 heterozygotes would achieve on-treatment platelet reactivity comparable to that seen with the standard 75 mg dose in wild-type individuals.[48] For patients carrying 2 loss-of-function alleles, even quadrupling the dose did not achieve bioequivalence. Similar data exist from the CLOVIS-2 trial for increasing the loading dose.[50] Data on dose escalation resulting in a difference in clinical outcomes is lacking.

Third-generation $P2Y_{12}$ inhibitors

Alternatively, one could use a third-generation $P2Y_{12}$ inhibitor such as prasugrel or ticagrelor. Prasugrel is also a thienopyridine that irreversibly binds the platelet $P2Y_{12}$ receptor to inhibit ADP-induced platelet aggregation.[51] In TRITON-TIMI 38, prasugrel compared with clopidogrel reduced the composite of cardiovascular death, MI, or stroke from 12.1% to 9.9% and reduced stent thrombosis from 2.4% to 1.1%.[52] However, prasugrel increased non-CABG-related TIMI major bleeding (from 1.8% to 2.4%), including fatal bleeding (from 0.1% to 0.4%).[52] Prasugrel was approved by the FDA for use in patients with ACS undergoing planned PCI. A genetic analysis within the TRITON-TIMI 38 trial found that loss-of-function polymorphisms in *CYP2C19* did not significantly affect active metabolite levels, platelet aggregation inhibition, or clinical cardiovascular event rates in individuals treated with prasugrel.[53] Therefore, the treatment benefit of prasugrel versus clopidogrel was greater in individuals harboring a loss-of-function variant than in those who did not.

The differential impacts of *CYP2C19* polymorphisms on clopidogrel versus prasugrel are likely mediated by differential involvement of esterases and the CYP450 system in the activation of clopidogrel and prasugrel. For clopidogrel, esterases shunt the majority of ingested clopidogrel to a dead-end inactive pathway, with the remaining prodrug requiring a 2-step CYP-dependent oxidation process to produce active clopidogrel metabolites; for prasugrel, esterases are part of the activation pathway and activation of prasugrel requires only a single CYP-dependent oxidative step.[53]

The other third-generation $P2Y_{12}$ inhibitor is ticagrelor, which is an active compound and not a prodrug, so it does not require hepatic CYP450-mediated activation. In the PLATO trial, ticagrelor compared with clopidogrel reduced the composite of vascular death, myocardial infarction, or stroke from 11.7% to 9.8%, as well as vascular death alone from 5.1% to 4.0%.[54] However, ticagrelor increased the rate of non-CABG-related Thrombolysis In Myocardial Infarction (TIMI) major bleeding (from 2.2% to 2.8%), but not fatal bleeding (0.3% in both arms).[54] Ticagrelor was approved by the FDA for use in patients with ACS. As would be expected, *CYP2C19* polymorphisms do not affect either the pharmacologic or clinical response to ticagrelor.[55] A genetic analysis within the PLATO trial found ticagrelor to be superior to clopidogrel in the treatment of ACS irrespective of *CYP2C19* polymorphism, but also found that the magnitude of benefit tended to be greater in carriers of loss-of-function alleles.[35]

Trials of pharmacogenetic testing

Trials specifically evaluating a strategy of pharmacogenetic testing are challenging to design. One issue is defining what treatment would be given in the control arm (assuming that in the arm with genotyping, loss-of-function carriers all would receive a third-generation $P2Y_{12}$ inhibitor). If the control arm receives clopidogrel, one must bear in mind that the pivotal trials that demonstrated the benefit of the third-generation $P2Y_{12}$ inhibitors over clopidogrel required 15 000-20 000 patients each. If only ~30% of the experimental arm is getting a third-generation $P2Y_{12}$ inhibitor, the sample size of such a trial would need to be far in excess of 20 000 patients. Conversely, if one designed the trial as a noninferiority trial and gave everyone in the control arm an (expensive) third-generation $P2Y_{12}$ inhibitor, then, again, the trial size would need to be far in excess of 20 000 patients to meet the typical FDA definition of noninferiority. Therefore, the genetic substudies in the randomized controlled trials of prasugrel and ticagrelor are likely the best data we will have and, as noted above, both suggest greater benefit of using a third-generation $P2Y_{12}$ inhibitor in patients who harbor a *CYP2C19* loss-of-function allele.

Guidelines and recommendations

The FDA prescribing information for Plavix notes that: "tests are available to identify a patient's *CYP2C19* genotype and can be used as an aid in determining therapeutic safety [and to] consider alternative treatment or treatment strategies in patients identified as *CYP2C19* poor metabolizers."[47] The American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) PCI guidelines do not mandate such testing, but rather simply note that

"Genetic testing might be considered to identify whether a patient at high risk for poor clinical outcomes is predisposed to inadequate platelet inhibition with clopidogrel."[56] With the superior clinical efficacy of the third-generation $P2Y_{12}$ inhibitors,[52,54] we generally favor their use. However, we recognize that clopidogrel continues to be used widely. In patients with ACS undergoing PCI in which the clinician is considering using clopidogrel, we believe that the current literature supports the use of prasugrel or ticagrelor when not contraindicated clinically in patients who carry a loss-of-function *CYP2C19* allele.[57]

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