

REVIEW ARTICLE

The Pharmacogenetics of Cytochrome P450 2C19 enzymes - Effects on Clopidogrel and Proton Pump Inhibitors

Yusmiati Liau^{1,*}, Dewi Muliaty¹¹Prodia Clinical Laboratory, Jl. Kramat Raya No.150, Jakarta, Indonesia

*Corresponding author. E-mail: yusmiati@prodia.co.id

Abstract

BACKGROUND: Cytochrome P450 (CYP) enzymes play important roles in human, including drug metabolism. CYP2 is the largest family of human CYP, with its sequence comprising almost one third of all CYP sequences, and responsible for the metabolism of approximately 2% of clinically administered drugs. One of the most important enzymes in this family is the CYP2C19 enzyme. The CYP2C19 gene is polymorphic, and the variation is common especially in the Asian population.

CONTENT: CYP2C19 is responsible for the metabolism of various drugs, including proton pump inhibitors (PPIs) such as omeprazole and lansoprazole, psychotropic drugs including diazepam and imipramine, anticonvulsants such as phenobarbital and mephenytoin, and the recently most studied the anti-platelet drug, clopidogrel, and many others. Drugs metabolized predominantly by this enzyme like clopidogrel and PPIs might be much affected by the genotype status of CYP2C19. Clopidogrel is a pro-drug requiring a group of enzymes to convert to its active form, particularly the CYP2C19. PPIs are metabolized to its inactive metabolites mainly by CYP2C19 in the liver. Some PPIs are inhibitor of CYP2C19 enzymes, and interaction of PPIs and clopidogrel has been widely studied.

SUMMARY: The association of CYP2C19 genotypes with the plasma level of active clopidogrel and platelet reactivity in individual taking this drug is well-established. Although conflicting results still exist for the association of CYP2C19 genotypes to the clinical outcomes of clopidogrel therapy, this effect seems to be consistent in patients receiving clopidogrel for coronary stents. Due to the interaction of certain PPIs and clopidogrel, the use of PPIs other than omeprazole is recommended, especially for patients taking

Abstrak

LATAR BELAKANG: Enzim sitokrom P450 (CYP) berperan penting pada manusia, termasuk salah satunya dalam metabolisme obat-obatan. CYP2 adalah famili CYP yang terbesar pada manusia, dengan panjang urutan gennya mencapai sepertiga dari seluruh gen CYP, serta bertanggungjawab terhadap metabolisme dari kurang lebih 20 % dari seluruh obat-obatan. Salah satu enzim yang paling penting dalam family CYP2 ini adalah enzim CYP2C19. Gen CYP2C19 bersifat polimorfik, dan variasi ini terutama umum dijumpai pada populasi Asia.

ISI: CYP2C19 bertanggungjawab terhadap metabolisme serangkaian obat, termasuk penghambat pompa proton (PPI) seperti omeprazole dan lansoprazole, obat psikotropika seperti diazepam dan imipramine, antikonvulsan seperti phenobarbital dan mephenytoin, dan yang paling banyak dipelajari sekarang ini, obat anti-platelet, clopidogrel, serta yang lainnya. Obat-obat yang dimetabolisme oleh enzim ini seperti clopidogrel dan penghambat pompa proton sangat dipengaruhi oleh status genotipe dari CYP2C19. Clopidogrel adalah suatu pro-drug yang memerlukan sekelompok enzim untuk terkonversi menjadi bentuk aktifnya, terutama enzim CYP2C19. PPI dimetabolisme menjadi metabolit inaktif terutama oleh enzim CYP2C19 di dalam hati. Beberapa jenis PPI merupakan inhibitor dari enzim CYP2C19, dan interaksi antara PPI dengan clopidogrel telah banyak dipelajari.

RINGKASAN: Hubungan antara genotipe CYP2C19 dengan kadar aktif clopidogrel dan reaktivitas clopidogrel pada individu yang mengkonsumsi obat ini sudah sangat jelas. Walaupun terdapat laporan yang saling bertentangan tentang hubungan antara genotipe CYP2C19 dengan data klinis dari terapi clopidogrel, efek ini tampaknya konsisten

dual anti platelet therapy of clopidogrel and aspirin.

KEYWORDS: pharmacogenetics, CYP2C19, proton pump inhibitors, clopidogrel

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pada pasien dengan stent koroner yang mendapatkan clopidogrel. Karena interaksi antara sebagian PPI dengan clopidogrel, penggunaan PPI selain omeprazole disarankan, terutama pada pasien yang mendapatkan terapi anti platelet gabungan, clopidogrel dan aspirin.

KATA KUNCI: farmakogenetik, CYP2C19, penghambat pompa proton, clopidogrel

Introduction

Pharmacogenetics is a term to describe how the gene sequence can affect the response of an individual to certain drugs or medication. One of the largest and most functional diverse genes is the Cytochrome P450 (CYP) enzymes.(1) Of this family, CYP2C19 especially plays significant role in Asian population due to its higher mutation rate. CYP2C19 is an enzyme essential for metabolism of some clinically important drugs such as clopidogrel, proton pump inhibitor (PPI) and some antidepressant.(2,3) FDA has approved new information on the label of clopidogrel regarding the genetic test for CYP2C19, as well as the potential interaction between clopidogrel and PPI, especially omeprazole.(4) In this review article, we discuss current evidence on the influence of CYP2C19 polymorphism to the outcomes of clopidogrel treatment, as well as the interaction with PPIs.

CYP

CYP enzymes family is one of the largest and most diverse superfamily, found in numerous living organism, from bacteria to humans, with remarkable functions.(1) In human, CYP plays various roles, including metabolism of drugs and foreign chemicals, metabolism of arachidonic acid and eicosanoids, metabolism of cholesterol and biosynthesis of bile acid, synthesis and metabolism of steroids, synthesis and metabolism of vitamin D3, hydroxylation of retinoid acid, the conversion of procarcinogens and promutagens to deleterious genotoxic compounds, and some still unknown functions.(1,5) This enzymes family was first named in 1961 when they were found to be bound to membranes within a cell (cyto) and contain a heme pigment (chrome and P) that absorbs light at a wavelength of 450 nm when exposed to carbon monoxide.(5,6) Human CYP superfamily comprises 57 functionally active genes, and 58 pseudogenes. This

number is much smaller compared those observed in plants and animals, for example there are 323 genes in rice (*Oryza sativa*) and 102 genes in mouse.(7)

A systematic nomenclature scheme for the CYP gene superfamily was published in 1987.(8) CYP proteins are conveniently arranged into families and subfamilies on the basis of percentage of amino acid sequence identity. Enzymes that share $\geq 40\%$ identity are assigned to a particular family designated by an Arabic numeral, whereas those sharing $\geq 55\%$ identity make up a particular subfamily designated by a letter.(5) But, this assignation also greatly depends on how the sequence clusters on a tree and not so much for the absolute percentage.(9) Enzymes with more than 97% identical are considered to represent alleles unless there is evidence to the contrary. A complete database of CYP can be found at <http://drnelson.uthsc.edu/CytochromeP450.html>.(7)

Human CYP Family

Human CYP enzymes are expressed on the smooth endoplasmic reticulum of cells throughout the body, with the highest concentrations to be found in the liver (hepatocytes) and small intestine.(10) They are grouped in 18 families and 43 subfamilies (7), with the first three families, CYP1, CYP2, and CYP3 being of the most importance (11).

CYP and Drug Metabolism

Metabolism of drugs, particularly the phase I oxidation is probably the most well-known function of CYP in humans. CYP superfamily that metabolizes these substances in the human body is almost exclusively the 3 families of CYP1, CYP2 and CYP3 family, and to a lesser degree CYP4.(1,5) Six enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5, metabolize 90% of drugs.(6)

CYP2 is the largest family of human CYP, with its

sequence comprising almost one third of all CYP sequences. (1) Of the CYP213 subfamilies, CYP2C enzymes are responsible for the metabolism of approximately 20% of clinically administrated drugs. Three enzymes from this subfamily are of most clinical importance, *i.e.* CYP2C8, CYP2C9 and CYP2C19.(1)

CYP2C19

CYP2C19 enzymes are responsible for the metabolism of various drugs, including PPIs such as omeprazole and lansoprazole, psychotropic drugs including diazepam and imipramine, anticonvulsants such as phenobarbital and mephenytoin. and the most studied recently the anti-platelet drug, clopidogrel, and many others.(2,3) CYP2C19 gene is located in chromosome 10 (10q24) and encodes a 490-amino-acid protein.(7,12) CYP2C19 and CYP2C9 genes are located in the same chromosome and the enzymes share a 91% structural similarity.(2)

All enzymes in the CYP2C subfamily are found to be genetically polymorphic.(13) Genetic polymorphism is defined as a mutation in the DNA sequence which is present in at least 1% of the population. The polymorphism can exist as deletion, insertion or single base substitution, or also known as Single Nucleotide Polymorphism (SNP). These SNPs can result in the change in the amino acid sequence, premature stop codon, or a splicing defect that can increase, decrease, or diminish the protein function.

In general, four phenotypes can be identified: poor metabolizers (PMs), who lack the functional enzyme; intermediary metabolizers (IMs), who are heterozygous for one loss of function allele or carry two alleles that cause reduced activity; extensive metabolizers (EMs), who have

two normal alleles; and ultrarapid metabolizers (UMs) who acquire the gain of function allele.(11)

The first polymorphism discovered in the CYP2C subfamilies was the well-known polymorphism of CYP2C19 in the metabolism of the anticonvulsant drug mephenytoin. (13) To date, at least 34 alleles of CYP2C19 have been identified (*1 to *34) with several subvariants within the same allele.(14) CYP2C19*1 is designated for the normal or wild-type allele and exhibits normal function (EM). From the numerous variants only a small number of variations give significance in terms of clinical and frequency.(14) The loss of function allele (CYP2C19*2) was first reported by de Morais *et al.* in 1994. They found that a single base pair (G>A) mutation in exon 5 of CYP2C19 (rs4244285), which creates an aberrant splice site is the major genetic defect that affects the metabolism of mephenytoin.(15) This mutation results in mRNA lacking the first 40 bp of exon 5, producing a premature stop codon and a truncated 234 amino acid protein lacking the heme-binding region. Later in the same year, the same group reported the second most common allele, the CYP2C19*3, with a change from G>A at position 636 of exon 4 of CYP2C19 (rs4986893), which creates a premature stop codon.(16) Both of these alleles result in a defective nonfunctional enzyme, with a phenotype of PM. CYP2C19*2 and CYP2C19*3 are the most frequent mutated alleles of this enzyme, and the frequency varies throughout different ethnics. In Asian population, these two polymorphisms account for more than 99% of all polymorphisms detected. (17,18)

Another polymorphism of clinical significance is the CYP2C19*17, which is a gain of function polymorphism, carrying -806 C>T and -3402 C>T mutation in the 5'-flanking region of the gene (12), and increase the transcription level (19). In the first report, this polymorphism cause

Table 1. Common loss of function polymorphisms of CYP2D6, CYP2C9, and CYP2C19 and their frequency in different races.

No.	Gene (Reference)	Alleles	Frequency		
			Asian	Caucasian	African American
1	CYP2D6 (25)	CYP2D6*3	<1%	<1-9%	<1%
		CYP2D6*4	<1-9%	>10%	<1-9%
		CYP2D6*5	1-9%	1-9%	<1-9%
		CYP2D6*6	<1%	<1%	<1%
2	CYP2C9 (22)	CYP2C9*2	0	13%	3%
		CYP2C9*3	4%	7%	2%
3	CYP2C19 (23,24,26)	CYP2C19*2	30.50%	1.20-1.40%	1.20-1.79%
		CYP2C19*3	8.50%	0%	0.70%

UM enzymes and results in lower level of omeprazole or mephenytoin, increasing the risk of treatment failure with these drugs.(20) Study correlating this mutation to the treatment with clopidogrel revealed that individual carrying this polymorphism had enhanced response to clopidogrel and an increased risk of bleeding.(21)

The polymorphism of CYP2C19 may be the most important in the Asian population due to its higher allelic frequency, compared to the other enzymes in this family, for example, CYP2C9, and CYP2D6. Table 1 shows the three enzymes that are important in human drug metabolism, together with their common polymorphisms and frequency in different races.(22-25) Our in-house data showed the allelic frequency of CYP2C19*2 and CYP2C19*3 as high as 24% and 4%, respectively.(26)

CYP2C19 - Effects on Clopidogrel

Clopidogrel is one of the most widely prescribed drugs in the world. It is used in the management of cardiocerebrovascular diseases, including acute coronary syndrome, myocardial infarction, stroke, and also in patients undergoing percutaneous coronary intervention. (3,4) Clopidogrel is often prescribed as dual therapy with aspirin, another anti-platelet agent.(3,27) Inter-individuality variation in the response of clopidogrel has been observed, and the term of clopidogrel resistance, nonresponders, poor responders is also known.(28) Clopidogrel is a thienopyridine derivative, and administrated as a pro-drug that requires metabolism by the hepatic enzymes to convert to its active form.(29)

After administered, clopidogrel is absorbed in the intestinal, regulated by ATP-binding cassette, sub-family B, member 1 (ABCB1) transporter located on the cell surface of intestinal epithelial cells. Once absorbed, approximately 85% of clopidogrel is metabolized into inactive forms, via hydrolysis in the liver by esterase. The remaining 15% will be converted into its active form (3), and then binds specifically and irreversibly to the platelet P2Y₁₂ Receptor, inhibiting adenosine diphosphate (ADP)-mediated platelet activation and aggregation (28).

The conversion of clopidogrel into its active form also occurs in the liver *via* a 2-step oxidation processes. These processes require CYP enzymes.(3) The first reaction is the conversion of clopidogrel into 2-oxo clopidogrel and the second is the conversion from 2-oxo clopidogrel to the active metabolites of clopidogrel (R-130964). The two processes are mediated by different enzymes. Kazui *et al.*, did a study to identify the enzymes involved in those two processes and they found that the first step was catalysed mainly by CYP2C19 (44.9%), followed by CYP1A2 (35.8%) and CYP2B6 (19.4%), and the second step involved another enzymes of CYP, namely CYP3A4 (39.8%), CYP2B6 (32.9%), CYP2C19 (20.6%), and CYP2C9 (6.76%).(29) This study's results confirm the importance of CYP2C19 enzyme in the metabolism of clopidogrel. Polymorphism of CYP2C19 is also the most thoroughly investigated among the others, in regard of the response of clopidogrel.

Polymorphisms of CYP2C19 have pharmacokinetic and pharmacodynamic effects upon intake of clopidogrel, and these effects were observed during loading doses (either 300 or 600 mg) as well as during the administration

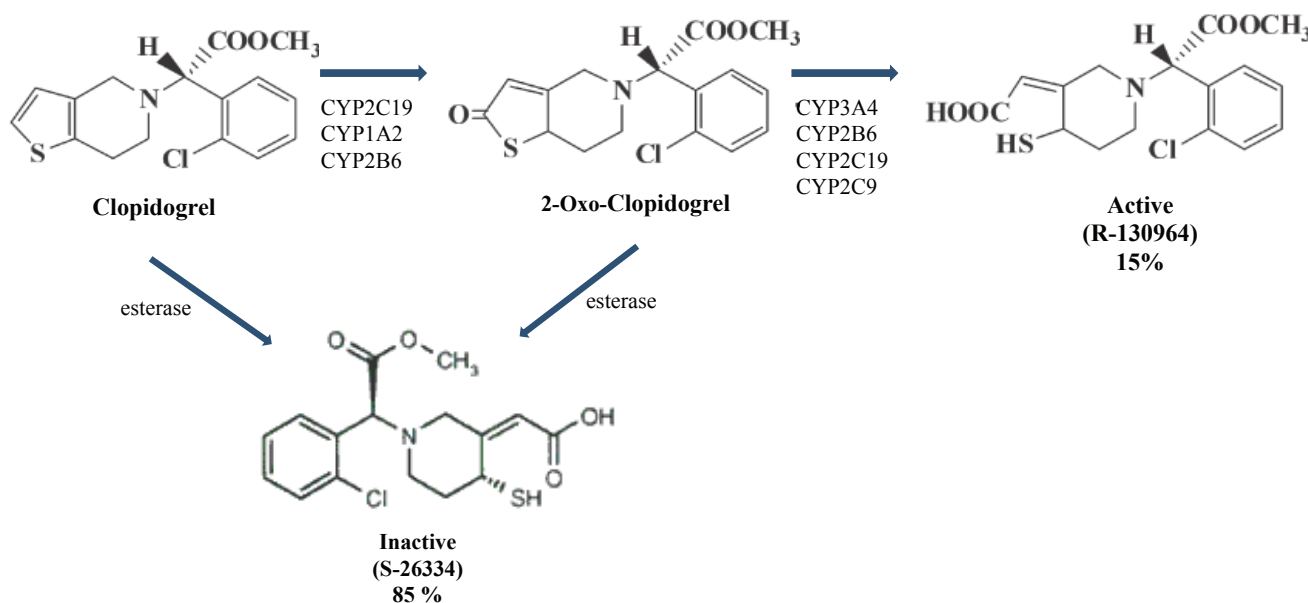


Figure 1. Conversion of Clopidogrel into its active metabolites.

of a maintenance dose.(30) A number of studies have noted the significance of CYP2C19 polymorphism in this phenomenon.(30-32)

It has been shown that CYP2C19 with even heterozygote loss of function allele (e.g. CYP2C19*1/*2) gives lower responsiveness to clopidogrel compared to the wild-type allele (*1/*1) ($p < 0.003$). (31) This polymorphism also correlates with the plasma level of active clopidogrel. (32) But to be noted, Hulot *et al.* have also found that 2 of 7 subjects in their study with the weakest response to clopidogrel have the wild-type genotype of CYP2C19. (31) Other studies also found that the polymorphism of CYP2C19 can only explain for 12-20% of total variability of clopidogrel response.(33,34) This indicates that response to clopidogrel is affected by other genetic or non-genetic factors. Several factors that have been reported are age, body mass index, diabetes, dyslipidemia, and others.(18)

Correlation of CYP2C19 Polymorphism with the Clinical Outcomes in Patients Taking Clopidogrel

Numerous studies have been conducted to investigate the effects of CYP2C19 polymorphism in clopidogrel response toward the clinical significance.(30,33,35-39) These studies did not only correlate this polymorphism with the plasma level of active clopidogrel or the platelet reactivity measured by some methods, but also correlate it further to the clinical outcomes, in short and long terms prospective studies.

Some studies have found the relative risk as high as 10 times for the loss of function CYP2C19 compared to wild-type (35), and others have also reported insignificant correlation (36,40).

Some meta-analyses and systematic review have been conducted to have a better look on the different results by different studies. Mega *et al.* in 2010 did a meta-analysis of 9 studies (cohort studies and clinical outcomes) in which clopidogrel was initiated in predominantly invasively managed patients. They concluded that there was a significant increased risk of the composite end point of cardiovascular death, myocardial infarction or stroke in carrier of 1 reduced-function CYP2C19 allele with the hazard ratio (HR) of 1.55 (95%CI: 1.11-2.17, $p=0.01$) and in carrier of 2 reduced-function with the HR of 1.76 (95%CI: 1.24-2.50, $p=0.02$). They also correlated it with stent thrombosis as the end point, and found the HR of 2.67 (95% CI: 1.69-4.22, $p<0.0001$) and HR of 3.95 (95%CI:

1.75-9.02, $p=0.001$) for carrier of 1 and 2 reduced-function CYP2C19 allele, respectively.(41)

Another meta-analysis by Sofi *et al.* in 2011 in 7 cohort studies, involving 8,043 patients that were followed for a period of time ranging from 6 months to 8.3 years also concluded a significant association between the CYP2C19*2 polymorphism and the increased risk of major adverse cardiovascular events with a relative risk (RR) of 1.96 (95%CI: 1.14-3.37, $p=0.02$), and with the increased risk of stent thrombosis with a RR of 3.82 (95%CI: 2.23-6.54, $p=0.0001$). (42)

Two other meta-analyses reported quite similar conclusion with each other. Bauer *et al.* in 2011 analyzed 15 studies that met their criteria of full length reports assessing the cumulative incidence of major adverse cardiovascular events or stent thrombosis over a follow-up period of at least a month in association with carrier status for the loss of function or gain of function CYP2C19 allele with coronary artery disease and a clinical presentation of acute coronary syndrome or stable angina pectoris who were taking clopidogrel. They reported the odds ratio (OD) of 1.77 (95%CI: 1.31-2.40, $p<0.001$) for stent thrombosis in carriers of at least one CYP2C19 loss of function allele versus non-carriers combining 9 studies, but after the adjustment for small study effect bias and replication diversity, the association was abolished. The OD of major adverse cardiovascular events for 12 studies combined was 1.11 (95%CI: 0.89-1.39, $p=0.36$). The OD of stent thrombosis in carriers versus non-carriers of at least one CYP2C19*17 gain of function allele for three studies combined was 0.99 (95%CI: 0.60-1.62, $p=0.96$), and the corresponding OD of major adverse cardiovascular events in 5 studies was 0.93 (95%CI: 0.75-1.14, $p=0.48$). (43)

Holmes *et al.* in 2011 analysed 32 studies, consisting of 6 randomized trial and 26 treatment only design. In treatment only studies, they found a higher risk of CVD events in individuals with 1 or more CYP2C19 alleles associated with lower enzyme activity with a relative risk of 1.18 (95%CI: 1.09-1.28), an absolute risk increase of 8-12 events per 1,000 individuals. To adjust the small study bias, they restricted the analysis to studies with 200 or more events, and found that the association was attenuated (RR 0.97; 95%CI: 0.86-1.09). But they found no association of CYP2C19 genotype with the CVD end point in effect modification studies. As a conclusion, they stated that there was an association between CYP2C19 genotype and clopidogrel responsiveness, shown by less clopidogrel metabolites, less platelet inhibition, and lower

risk of bleeding, but there was no significant association of genotype with cardiovascular events.(44)

In a response to the article above, Shuldiner *et al.* confronted the small study bias explored by the meta-analysis. They argued that the small studies pointed tent to be those including a higher proportion of patient with stents, in whom clopidogrel is more likely to be effective. This is also consistent with the meta-analysis by Mega *et al.* (41), involving studies predominantly with patients receiving percutaneous coronary intervention (PCI), which found a significant effect. Shuldiner also argued that an alternative interpretation of the meta-analysis by Holmes is that CYP2C19 genotype is an important predictor of adverse cardiovascular outcome for patient with coronary stent, and that it may be less important for patient taking clopidogrel for other indication, particularly those in which the drug has a smaller overall effect on outcomes (45).

Genetic Factors other than CYP2C19

As has been stated, CYP2C19 genetic variation can only explain for about 12-20 % of all variation in clopidogrel response. Other genes have been studied including ABCB1/MDR1 gene, other CYP enzymes (CYP3A4, CYP3A5), P2Y12 receptor, and most recently Paraoxonase-1 (PON-1). Intestinal absorption of the pro drug clopidogrel is limited by an intestinal efflux pump P-glycoprotein coded by the ABCB1 gene. There are several reports of an increase in the hazard ratio for an outcome event among patients carrying the ABCB1 variant allele (genotype CT or TT) as compared with the wild-type allele (CC).(36,46) ABCB1 T allele is associated with increased intestinal efflux possibly mediated by higher P-glycoprotein expression, and a reduced concentration of active drug metabolite.(46) On the other hand, another study by Wallentin reported contrasting result, with a higher rate of primary events for the ABCB1 3455 CC genotype.(47)

As the conversion of clopidogrel to its active form is also mediated by enzymes other than CYP2C19, it was suspected that other enzymes also contribute to the variation of clopidogrel response. A study reported that CYP3A4 IVS10+12G/A modulated platelet activation, and thus may contribute to clopidogrel response variability.(48) Cuisset *et al.* reported that CYP3A4*1B or CYP3A5*3 did not correlate with ADP-induced platelet aggregation.(49) Reports of the role of P2Y12 receptor polymorphism have tended to be not associated with a risk of adverse outcome

(36, 48).

The most recently reported gene affecting the efficacy of clopidogrel is the PON-1. This enzyme is regarded as the crucial enzyme for clopidogrel bioactivation, with its common Q192R polymorphism determining the rate of active metabolite formation. PON-1 QQ192 homozygous individuals showed a considerably higher risk than RR192 homozygous individuals of stent thrombosis, lower PON-1 plasma activity, lower plasma concentrations of active metabolite and lower platelet inhibition.(50) This report has been refuted by two recent studies, re-confirming the role of CYP2C19, and not PON-1 in the clopidogrel responsiveness.(51,52) It should also be noted that the genome-wide association study on clopidogrel efficacy did not identify SNPs surrounding or including the PON-1 locus, only the area surrounding the CYP2C enzyme cluster was associated with diminished clopidogrel response.(33)

FDA recommendation on the label of Clopidogrel (Plavix)

Responding to the studies reporting the significance of CYP2C19 polymorphism to the response of clopidogrel, FDA has so far made 3 recommendations on the label of the drug.(4) Firstly in May 2009, FDA simply noted that “poor metabolizer status is associated with diminished response to clopidogrel” and that “the optimal dose for poor metabolizers has yet to be determined”. The second revision in 2009 advised avoiding the use of clopidogrel “in patients with impaired CYP2C19 function due to known genetic polymorphisms or due to drugs that inhibit CYP2C19 activity” and added additional information about the interaction of clopidogrel and omeprazole. In the last revision in March 2010, FDA approved a new label for clopidogrel with a “boxed warning” stating “There is reduced effectiveness in patients who are poor metabolizers, tests are available to identify patients with genetic polymorphisms, and that alternative treatment strategies should be considered in poor metabolizers of the drug”.(18)

Implementation in Clinical Practice

Although the association of genetic polymorphism of CYP2C19 with the response of clopidogrel is well-established, and there are numbers of studies confirming the association to the clinical outcomes, how the tests of

the genetic polymorphism should be prescribed in the routine practice is still an unsolved problem. There are still questions on what patients should be tested, when to test, and what are the alternative treatment for those detected to be PM. Increasing the dose of clopidogrel in heterozygote loss of function carrier to 225 mg can achieve the level of platelet reactivity similar to that seen in standard 75 mg dose in non-carrier, but in homozygotes, increase to 300 mg daily did not result in comparable degrees of platelet inhibition. More over this study only measured the platelet reactivity, and did not measure the higher dose on the clinical outcomes.(53) Several studies have reported that prasugrel another thionopyridines is not affected by the CYP2C19 (54-56), and can be an alternative for the PMs, but this drug is also related to more risk of bleeding (57). Ticagrelor has been also proposed as an alternative to clopidogrel, which is not affected by the CYP2C19 genotypes.(47,58) The genetic polymorphism test was reported not yet to offer an opportunity to optimize treatment given the current state of knowledge (59), but the other reported that genotype-guided antiplatelet therapy between clopidogrel and prasugrel may be more cost-effective and may provide more clinical value due to fewer adverse outcomes.(60)

CYP2C19 - Effects on PPIs

PPIs are used to treat acid-related disorder like the gastro oesophageal reflux disease or oesophagitis/gastritis and also in eradication of *Helicobacter pylori* infection together with antibiotics.(61) Omeprazole was the first PPI introduced in the market, followed by pantoprazole, lansoprazole and rabeprazole. Though these PPIs share the core structures benzimidazole and pyridine, their pharmacokinetics and pharmacodynamics are a little different (62).

PPIs are metabolized in the liver by CYP, specifically CYP2C19 and CYP3A4. Omeprazole for example is mainly metabolized to 5-hydroxyomeprazole by CYP2C19 and to omeprazole sulphoneby CYP3A4.(63) Ranzoprazole is an exception and reported to be only partly CYP2C19 mediated.(64)

Different enantiomer of the drugs also bind the CYP enzyme with different affinity. R-omeprazole was more sensitive to CYP2C19 while S-omeprazole was less sensitive. Therefore, S-omeprazole provided better plasma level of the drug. Like omeprazole, lansoprazole also was extensively metabolized by CYP2C19 and CYP3A4. (62) Individuals with PM CYP2C19 enzymes exhibit a 3 to

10 folds higher area under the plasmic concentration curve (AUC) than homozygote EM (HomEM), while heterozygote EM (HetEM) exhibit a 2 to 3 folds higher AUC.(62)

Evidence of the association between CYP2C19 polymorphism and the therapy outcomes of PPIs has been accumulated (65-68), but lack consistency in terms of the association with different PPIs used (64). At least, four meta-analyses have been conducted to more clearly understand this association.

In 2006, a meta-analysis including twenty eight arms from seventeen papers reported that omeprazole but not lansoprazole or rabeprazole has different treatment efficacy based on the CYP2C19 enzyme status. Omeprazole as a component of either in dual or triple therapy regiment gives better outcome in PM versus HomEM with OD of 4.03 (95%CI: 1.997-8.28, p=0.0001), and also in PM vs. HetEM with OD of 2.24 (95% CI: 1.09-4.61, p=0.03).(69)

Another meta-analysis performed in 2008 involving 20 articles reported significant higher eradication rate for omeprazole and lansoprazole with in PM vs. HomEM (OD of 4.28, p=0.0005 for omeprazole and OD of 3.06, p=0.001 for lansoprazole), and also in HetEM vs. HomEM (OD of 3.22, p<0.0001 for omeprazole and OD of 1.95, p=0.04 for lansoprazole). Ranzoprazole was not affected by the CYP2C19 genotypes.(70)

In 2012, McNichol analyzed 35 studies comprising 5,998 patients and reported that CYP2C19 genotype did not influence omeprazole, lansoprazole, pantoprazole or the newer generation of PPIs, esomeprazole or rabeprazole. But in CYP2C19 EM, they found that new generation of PPIs might be more effective.(71)

Another meta-analysis was reported in 2013 by Tang *et al.* In this newer meta-analysis of 16 randomized controlled trials involving 3,680 patients, they found that the metabolizer status affects the efficacy of omeprazole, with OD of 0.329 (95%CI: 0.195-0.553) for HomEM vs. HetEM and 0.232 (95%CI: 0.105-0.515) for HomEM vs. PM, and lansoprazole with OD of 0.692 (95% CI: 0.485-0.988) for HomEM vs. HetEM and 0.441 (95%CI: 0.252-0.771) for HomEM vs. PM. No difference was observed for HetEM vs. PM. In regards to rabeprazole or esomeprazole, no class effect of CYP2C19 genotypes was seen.(64)

Esomeprazole is a pure S-isomer of omeprazole, and thus is less sensitive to CYP2C19, and this might explain why the efficacy of this drug is not affected by the enzyme. Similarly, rabeprazole is metabolized to thioether-rabeprazole mainly via a non-enzymatic pathway with minor involvement of CYP2C19, which is consistent with

the results of several meta-analyses.(64,69,70)

Interaction between Clopidogrel and PPIs

PPIs are often prescribed together in patients receiving dual anti platelet therapy to prevent the gastrointestinal (GI) bleeding. In 2011 American College of Cardiology Foundation (ACSF) / American Heart Association (AHA) / Society for Cardiovascular Angiography and Interventions (SCAI) Guideline for PCI, PPIs should be used in patients with a history of prior GI bleeding who require Dual Anti Platelet Therapy (DAPT) (I).

This guideline also states that use of PPIs is reasonable in patients with an increased risk of GI bleeding (*e.g.*, advanced age, concomitant use of warfarin, steroids, non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* infection) who require DAPT. (IIa), and routine use of a PPI is not recommended for patients at low risk of GI bleeding, who have much less potential to benefit from prophylactic therapy (III, no benefit).(72)

Some class of PPIs (omeprazole, lansoprazole) are metabolized mainly by CYP2C19 and some class of PPI like omeprazole and esomeprazole are inhibitors of CYP2C19 (73-75) with omeprazole being the most potent (73). Lansoprazole, pantoprazole, and rabeprazole are reported to be not inhibiting CYP2C19.(74)

The significant association of this inhibition effect of omeprazole to the attenuated inhibition of platelet aggregation when clopidogrel and omeprazole are concomitantly used, has been reported by numerous studies. (76-78)

Furuta *et al.* also reported that besides omeprazole, lansoprazole and rabeprazole also affect inhibition of platelet activity to some degree (76), but other reports showed contrasting results for PPIs other than omeprazole. (77,79)

Several reports have been less consistent regarding the clinical effect of this interaction. Some studies have reported a significant association of concomitant use of clopidogrel and PPIs with higher adverse outcomes (80,81), while others reported conflicting results (82-85).

Another study found only a significant association of omeprazole with the increased rate of hospitalization, while not the case with other PPIs.(79) And two studies focusing on patients receiving PCI also could not find an association between use of PPI and clopidogrel with higher adverse outcomes.(86,87)

Two review articles have suggested some possibility that may lead to significant results by some groups but not by the others including higher comorbidities in the group using PPIs, age bias, and low quality design of study that did not adjust to the confounding factors as in the significant result groups.(88,89) So far, the only existing randomized controlled double-blind multicenter trial is the clopidogrel and the optimization of gastrointestinal events trial (COGENT), which involved 3,878 patients with acute coronary syndrome (ACS) or undergoing PCI. This study showed no difference in the occurrence of cardiovascular events in clopidogrel+omeprazole group from that in the clopidogrel only group, and that the omeprazole significantly decreased the bleeding risk.(84)

However, two other review articles questioned the power of this study result to exclude the clinical interaction between omeprazole and clopidogrel. Tarnawski pointed out the wide CI around the HR of cardiovascular events and a possibility of population bias, as 94 % of the COGENT subjects are white.(88) Fernando *et al.* also raised some concern regarding the low events of ACS in this study.(89)

In one meta-analysis on 23 studies covering 93,278 patients, the authors pointed out that the positive significant association was reported by observational studies, while propensity matched and randomized trials failed to prove such association between concomitant use of PPIs and clopidogrel with the cardiovascular risk (90). A similar result reported by Lima *et al.*, with low quality design study reported a significant association, while none of the moderate or high quality study did (91).

Another meta-analysis by Siller-Matula concluded a significant association with major cardiovascular events, but not with mortality. PPIs were also found to associate with lower risk of bleeding. (92). These three meta analyses pointed out the same point that there was high grade of heterogeneity among the studies, including different populations, outcomes assessed, drug exposure methods and study quality, that might lead to some bias, unmeasured confounding factors, and inconsistent results (90-92). Lima *et al.* also analyzed that there was inverse correlation between study quality and a reported statistically positive interaction. (91)

Despite inconsistent results, on the label of Plavix, FDA has discouraged the use of PPIs (especially omeprazole and esomeprazole) concomitantly with clopidogrel, and recommend the use of PPIs with lesser inhibitory effect to the CYP2C19 (dexlansoprazole, lansoprazole and pantoprazole).(4)

Conclusion

CYP2C19 is an important enzyme that metabolizes a great number of drugs. Polymorphism of this enzyme is common especially in the Asian population. Drugs metabolized predominantly by this enzyme like clopidogrel and PPIs might be much affected by the genotype status of CYP2C19. Although conflicting results exist regarding for the association of CYP2C19 genotypes with the clinical outcomes of clopidogrel therapy, this effect seems to be consistent in patients receiving clopidogrel for coronary stents, with individuals carrying the loss of function allele having higher risk of future cardiovascular events as well as stent thrombosis. Interaction of PPIs and clopidogrel has been widely studied, and the use of PPIs other than omeprazole is recommended, especially for patients taking dual anti platelet therapy of clopidogrel and aspirin.

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