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The impact of genetic polymorphisms of drug metabolizing enzymes on the pharmacodynamics of clopidogrel under steady state conditions

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ABSTRACT

Clopidogrel is an antiplatelet drug that requires biotransformation steps to its active metabolite via cytochromes P450 (CYP), particularly CYP2C19 and CYP3A5 as well as paraoxonase-1 (PON1). The impact of *CYP3A5* and *PON1* genetic polymorphisms on the response of this drug is unclear. This study aimed to elucidate the degree of genetic polymorphisms of key drug metabolizing enzymes on the antiplatelet effect of clopidogrel. Thirty-five healthy subjects were treated with 75 mg/day clopidogrel for 7 days and serial blood samples were collected for measurement of antiplatelet effect using whole blood impedance aggregometry and VerifyNow® P2Y12 methods. The areas under the antiplatelet effect–time curves, maximal and minimal antiplatelet effects of clopidogrel obtained from both methods were significantly different among subjects with different *CYP2C19* genotypes. In contrast, these pharmacodymamic parameters measured by both methods of subjects with different *PON1* or *CYP3A5* genotypes were not significantly different. Among the heterozygous *CYP2C19*2* subjects, all pharmacodynamic parameters measured by whole blood impedance aggregometry were significantly different between subjects with different *CYP3A5*3* genotypes. Our data suggests that *CYP2C19* genetic polymorphism play a major role in the clopidogrel response, however, the impact of *CYP3A5* genetic polymorphism, may be pronounced in the subjects who carried the loss-functional allele of *CYP2C19*.

1. Introduction

Clopidogrel is an antiplatelet aggregation drug that acts by inhibiting the binding of adenosine 5-diphosphate (ADP) to its platelet P2Y12 receptor that subsequently leads to the inhibition of ADP-mediated activation of the GPIIb/IIIa complex [1]. This drug is commonly used for treatment and prevention of atherothrombotic events in patients who have cardiovascular and cerebrovascular diseases. Current available data, however, has shown that about 5%–44% of patients who were treated with the conventional dose of clopidogrel do not display an adequate antiplatelet aggregation response [2]. Due to the fact that clopidogrel is a prodrug and requires a biotransformation process before it can exert antiplatelet aggregation activity, the impact of genetic drug metabolizing enzyme polymorphisms on the clinical response of clopidogrel is of particular interest.

Clopidogrel requires two biotransformation steps for conversion into an active thiol derivative metabolite (Fig. 1). The first biotransformation step is catalyzed by several cytochrome P450 (CYP) enzymes including CYP2C19, CYP1A2 and CYP2B6 whereas the second step is mediated by CYP3A4/5, CYP2B6, CYP2C19 and CYP2C9 as well as paraoxonase-1 enzyme (PON1) [3]. Of the CYP enzymes involved in the bioactivation of clopidogrel, CYP2C19 was found to play a key role because it contributes to both sequential bioactivation steps [4]. To date, more than 20 variants of *CYP2C19* have been reported and the "loss-of-function" allele, *CYP2C19*2* is the most common variant allele. *CYP2C19*2* has been reported to be associated with an attenuated response to clopidogrel in healthy

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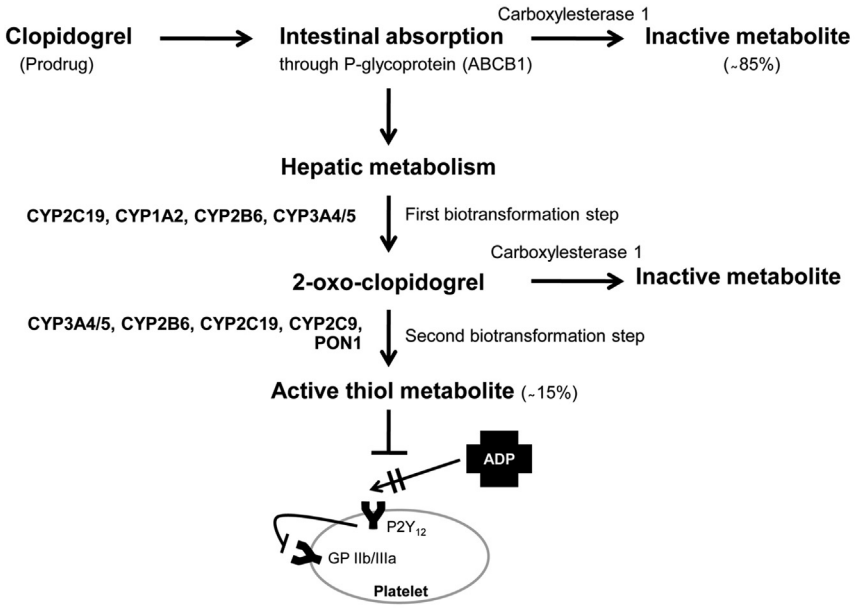

Fig. 1. The metabolic pathways of clopidogrel and its antiplatelet effect [3].

subjects [5–7] and patients who carried this variant allele are at higher risk of atherothrombotic events [8–11]. In addition, the conversion of 2-oxo-clopidogrel to its thiol active metabolite, a second bioactivation step of clopidogrel, is substantially catalyzed by CYP3A enzymes [4]. Although *CYP3A4* is the predominant CYP3A form which is expressed in liver, the polymorphic *CYP3A5* may contribute up to 50% of the CYP3A activity in 30%–50% of individuals [12]. *CYP3A5*3*, the single most common variant allele in Asian populations, is strongly correlated with a decreased CYP3A5 activity, whereas the *CYP3A5*1* allele is correlated with high CYP3A5 activity [12]. Unlike *CYP2C19*2*, the effect of CYP3A5 on the response to clopidogrel is still controversial. The clinical significance of the *CYP3A5* genotype in determining the risk of atherothrombotic events has been reported in clopidrogrel treated Korean patients undergoing percutaneous coronary intervention (PCI) in which atherothrombotic events occurred more frequently among patients with the *CYP3A5*3* genotype [13]. Moreover, it has been shown in healthy volunteers that the antiplatelet activity of clopidogrel was apparently higher in the *CYP3A5*1* genotype group than in the *CYP3A5*3* genotype group [13]. In contrast, previous studies in a French population and an Iranian population undergoing PCI showed that *CYP3A5*3* polymorphism did not influence the antiplatelet activity of clopidogrel [14,15].

Apart from CYP enzymes, an esterase PON1 has been reported as the rate-limiting enzyme for the second bioactivation step of clopidogrel to its active thiol metabolite [3]. The common genetic variant of *PON1*, glutamine (Q)/arginine (R) at position192 (*Q192R*) which mostly accounts for the wide inter-individual variation in serum paraoxonase activity has been reported to be associated with clopidogrel response. The *PON1 QQ192* homozygous individuals showed considerably lower PON1 plasma activity, lower plasma concentrations of clopidogrel active metabolite and lower platelet inhibition as well as at the higher risk of stent thrombosis than *RR192* homozygous individuals [16,17]. Some recent studies reported that the *PON1 Q192R* genetic polymorphism did not influence the platelet response to clopidogrel or the cardiovascular events in clopidogrel-treated patients [8,9].

Apart from genetic polymorphisms of these drug-metabolizing enzymes, other non-genetic factors including lack of drug compliance, drug–drug interactions, clinical factors such as obesity, insulin resistance and the nature of the cardiovascular diseases may also contribute to the variability of the clopidogrel response [8,18]. The real impact of the genetic polymorphisms of *CYP2C19*, *CYP3A5* and *PON1* may be therefore influenced by these non-genetic factors. It should be noted that almost all of the previous studies reported so far were based on a single platelet functional test or single genetic polymorphisms of these drug metabolizing enzymes. A recent study reported that the effect of influencing factors on antiplatelet activity of clopidogrel may depend on the assay systems used for evaluation of platelet function [19]. Therefore, a study in healthy volunteers to elucidate the degree of crucial genetic polymorphisms of all key drug metabolizing enzymes relevant to clopidogrel metabolism on the pharmacodynamic parameters of clopidogrel under steady state conditions using two different platelet function tests was conducted.

2. Methods

2.1. Study subjects

Thirty-five healthy Thai male volunteers with different *CYP2C19*, *CYP3A5* and *PON1* genotypes who met the following criteria were enrolled into the study: age between 18 and 45 years; BMI within 18–25 kg/m^2; no clinically significant abnormalities, as confirmed on medical history, physical examination and clinical laboratory analysis. Subjects were excluded if they had a history or evidence of hepatic, renal, gastrointestinal, hematologic abnormalities such as thrombocytopenia (platelets < 100,000 cells/mm^3); anemia (hemoglobin < 10 g/dL); any other acute or chronic diseases or hypersensitivity to clopidogrel. No medications, tobacco, herbal medicine, alcohol, grapefruit juice, or beverages containing caffeine were permitted two weeks before and during the study period. The study protocol was approved by the Khon Kaen University Ethics Committee for Human Research, Khon Kaen University, Thailand with the Declaration of Helsinki and written informed consents were obtained from all participants.