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Impact of CYP2C19 Polymorphism and Proton Pump Inhibitors on Platelet Reactivity to Clopidogrel and Clinical Outcomes Following Stent Implantation

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ABSTRACT

Background: The response to clopidogrel, and some kind of the drug interaction are multifactorial.
Methods and Results: We enrolled 174 consecutive patients and determined CYP2C19 genotypes, measured platelet aggregation, and assessed the relationship between CYP2C19 genotype and platelet reactivity 24 hours after clopidogrel administration, and the risk of cardiovascular events over 18 months follow-up. A sub analysis examined the impact of rabeprazole, a proton pump inhibitor (PPI) less affected by CYP2C19.
The CYP2C19 genotype was extensive metabolizer (EM) in 36%, intermediate metabolizer (IM) in 45%, and poor metabolizer (PM) in 19%. Platelet reactivity was significantly lower in the EM group than in the IM and PM groups (EM, IM, PM: 3560 ± 1404, 4203 ± 1302, 5084 ± 1007 AU•min, $P < 0.05$). The cardiovascular event rate was higher in the IM and PM groups than in the EM group (12.7% and 12.5% vs 1.6%; Hazard ratio for IM 10.6, $P = 0.029$; for PM 11.3, $P = 0.040$). No differences were seen between patients taking ($N = 50$) and not taking ($N = 124$) rabeprazole in residual platelet aggregation (4407 ± 1360 vs 4048 ± 1394, AU•min, $P = 0.2782$), or in cardiovascular events (10.0% vs 8.1%, HR 0.97, $P = 0.97$).
Conclusions: CYP2C19 genotype is associated with an increased risk of cardiovascular events following stent implantation in Japanese patients.

Introduction

Treatment failure with clopidogrel can be due to various reasons [1,2], including polymorphism in the gene encoding the Cytochrome P450 2C19 enzyme (CYP2C19). Clopidogrel is a prodrug that has to be converted to an active metabolite [3]. CYP2C19 is a key factor in this activation process [4], and the presence of CYP2C19 loss-of-function alleles is associated with reduced clopidogrel responsiveness in patients with coronary heart disease (CHD) [5]. The U.S. Food and Drug Administration (FDA) issued a Drug Safety Communication for Clopidogrel [6] about attenuated effectiveness of clopidogrel in patients with impaired ability to convert the drug to its active form. This warning was based on recognition that the antiplatelet effect of clopidogrel depends primarily on its activation by CYP2C19. Patients with decreased CYP2C19 function due to genetic polymorphism metabolize clopidogrel poorly, and have higher rates of cardiovascular events following acute coronary syndrome (ACS) and percutaneous coronary interventions (PCIs) than patients with normal CYP2C19 function. The warning also notes that alternative treatment strategies should be considered in poor metabolizers of clopidogrel. The prevalence of CYP2C19 loss-of-function alleles is much higher in East Asians, including Japanese, than in people in Western countries [7]. Japanese patients in particular require consideration of their response to clopidogrel treatment.

Another consideration is the possible drug interaction between clopidogrel and proton pump inhibitors (PPIs). The American College of Cardiology Foundation (ACCF), American College of Gastroenterology (ACG) and American Heart Association (AHA) recommended at their 2008 conference that a PPI should be administered to patients during dual antiplatelet therapy with clopidogrel and aspirin, for prophylaxis in patients with a high risk of upper gastrointestinal bleeding [8].

Abbreviations: CYP2C19, Cytochrome P450 2C19 enzyme; CHD, coronary heart disease; FDA, Food and Drug Administration; ACS, acute coronary syndrome; PCIs, percutaneous coronary interventions; PPIs, proton pump inhibitors; ACCF, American College of Cardiology Foundation; ACG, American College of Gastroenterology; AHA, American Heart Association; PCR, Polymerase chain reaction; RFLPs, restriction fragment length polymorphisms; EM, extensive metabolizers; IM, intermediate metabolizers; PM, poor metabolizers; ADP, adenosine diphosphate.

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Although PPIs are widely used to reduce the risk of gastrointestinal bleeding in patients receiving dual antiplatelet therapy, several studies have indicated that concomitant use of clopidogrel and a PPI is associated with reduced antiplatelet efficacy of clopidogrel, and increased adverse clinical outcomes after stent placement in patients with ACS [9–13]. Both clopidogrel and PPIs are metabolized by CYP isoenzymes, but to varying degrees, and clopidogrel is metabolized intoactive and PPIs into inactive metabolites [14,15]. The mechanism of the negative interaction between clopidogrel and PPIs may be due in part to competitive inhibition of the CYP2C19 enzyme [16]. While other PPIs, especially omeprazole, are mostly metabolized by CYP2C19, rabeprazole is mostly metabolized by non-enzymatic pathways, and shows the least potent inhibition of CYP2C19 *in vitro* [15]. Given that there are differences in the contribution of CYP isoforms to the different metabolic pathways, we hypothesized that not all PPIs have a negative interaction with clopidogrel. Accordingly, we investigated whether CYP2C19 polymorphism or concomitant use of rabeprazole and clopidogrel is associated with on-treatment platelet reactivity and clinical outcomes in Japanese patients with CHD following stent implantation.

Materials and Methods

Study Populations

We registered 197 subjects out of 237 consecutive patients recorded on the PCI list between December 2008 and January 2010. The exclusion criteria were: 1) written informed consent could not be obtained from the subject or their family; 2) already taking either clopidogrel or aspirin; 3) on anticoagulants, or antiplatelet agents other than clopidogrel or aspirin; and 4) already taking anticoagulants, antiplatelet agents or PPIs routinely before admission. In the end, we enrolled 174 patients following stent implantation, taking dual antiplatelet therapy with 100 mg/day aspirin and 75 mg/day clopidogrel as a maintenance dose after a loading dose of clopidogrel 300 mg, in this prospective single center study with 18 months follow-up.

Subjects were assigned to 1 of 2 groups, administered rabeprazole (10–20 mg/day, n = 50) and not administered rabeprazole (n = 124). Rabeprazole was started on the same day as clopidogrel loading. The decision to administer rabeprazole or not was at the discretion of the treating physician, mainly on the basis of a prior history of upper gastrointestinal ulcer or bleeding, or the presence of upper gastrointestinal symptoms, such as heartburn or epigastric pain. We checked compliance with all test drugs.

Genotyping

Genomic DNA was extracted from whole blood using a DNA Extractor WB kit (Wako Pure Chemical Industries Ltd, Osaka, Japan) using a protocol modified from Richards et al. [17]. Polymerase chain reaction (PCR) restriction fragment length polymorphisms (RFLPs) for CYP2C19*2 (681G > A) and CYP2C19*3 (636G > A) were performed as described previously [18,19]. CYP2C19*2 and *3 are considered to account for >99% of alleles generating the null-activity enzyme protein in the Japanese population [19]. CYP2C19 genotypes were therefore classified into three phenotypes: 1) extensive metabolizers (EM) carrying normal function alleles (CYP2C19*1/*1), 2) intermediate metabolizers (IM) carrying one loss-of-function allele (*1/*2, *1/*3), and 3) poor metabolizers (PM) carrying two loss-of-function alleles (*2/*2, *2/*3, *3/*3).

Measurement of Residual Platelet Reactivity

Platelet function tests were performed 24 hours after administration of a loading dose of 300 mg clopidogrel, followed by a 75 mg daily maintenance dose. Platelet aggregation was measured as follows. Whole samples of blood were obtained using a glass tube containing 0.38% sodium citrate solution. Platelet-rich plasma was prepared by centrifugation at 3000 g at room temperature for 15 min, followed by centrifugation at 10000 g at room temperature for 10 min to separate platelet-poor plasma. Aggregation in platelet-rich plasma induced by 20 μmol/L adenosine diphosphate (ADP; Chrono-Log) was measured using a light transmission aggregometer (MCM HEMA TRACER 313; PAM12C, LMS Inc. Japan), where the degree of light transmission of platelet-rich plasma was defined as 0% of the aggregation rate, and the cognitive platelet-poor plasma as 100%. Test time was 10 min. Residual platelet reactivity was defined as the area under the platelet aggregation curve, which was used to express the aggregation response over the measured time (aggregation units•min, AU•min) [20,21].

We used on-treatment platelet reactivity directly, not inhibition of platelet aggregation calculated as the percentage decrease in the relative maximal platelet aggregation from the baseline. Observations of clopidogrel response that rely on baseline platelet reactivity have recently been shown to be less reliable predictors of ischemic risk than post-treatment platelet reactivity [2,22,23]. The area under the aggregation curve (AU•min) is more sensitive and precise than maximal platelet aggregation calculated from the percentage of inhibition. Accordingly, we used the area under the aggregation curve as a measure of on-treatment platelet reactivity during antiplatelet therapy.

Clinical Outcomes

The study endpoints were residual platelet reactivity and cardiovascular death, nonfatal myocardial infarction, unstable angina, ischemic stroke, or coronary revascularization to new lesions. Cardiovascular events were surveyed by phone calls to subjects and their families, followed by a review of medical records, electrocardiography, echocardiography, and cardiac enzyme levels. Cardiovascular death was defined as death due to myocardial infarction, congestive heart failure, or documented sudden cardiac death. We used the universal definition of myocardial infarction in this study [24]. The diagnosis of ischemic stroke was made if a subject had clinical and radiological evidence of stroke without intracranial hemorrhage. For subjects experiencing more than 2 acute events, only the first event was considered in the analysis. Revascularization therapy based only on angiographic data, including PCI-mediated restenosis, was not counted as a cardiovascular event. Subjects were followed up every month as outpatients for an 18 months period or to end point.

Statistical Analyses

We analyzed platelet reactivity and clinical outcomes according to CYP2C19 genotype and rabeprazole use. Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequencies and percentages. Statistical analyses were performed exclusively by an independent statistician. We compared subject baseline characteristics by genotype and by rabeprazole use using the χ^2 test for categorical variables, and one-way analysis of variance (ANOVA) for continuous variables as appropriate. The time to first cardiovascular event was then compared between genotypes and using or not using rabeprazole for log-rank tests. Cumulative incidence rates were calculated and expressed as number of cardiovascular events. We used Cox regression analyses to calculate the hazard ratio to experience a first cardiovascular event according to genotype and taking or not taking rabeprazole. We constructed Kaplan-Meier curves for cumulative event-free survival for the endpoints. Comparisons are expressed as multivariate hazard ratios and 95% Confidence intervals (CIs). Predictors identified through univariate analysis, and other variables considered likely to have important prognostic value, were tested in a multivariable, stepwise, forward Cox proportional-hazards model for association with cardiovascular outcomes during the 18 month follow-up period. A p-value <0.05 was regarded as significant. All statistical analyses were conducted using SAS 9.1 (SAS Institute Inc., Cary, North Carolina).