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Original article

Patients with both CYP2C19 loss-of-function allele and peripheral endothelial dysfunction are significantly correlated with adverse cardiovascular events following coronary stent implantation

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ABSTRACT

Background: There is some controversy regarding the effect of CYP2C19 polymorphism on clinical outcome in patients receiving dual antiplatelet therapy (DAPT). Peripheral endothelial dysfunction has recently been reported to predict adverse cardiovascular events. We hypothesized that CYP2C19 loss-of-function (LOF) allele carriers with peripheral endothelial dysfunction had worse prognosis. The aim of this study was to evaluate an additive effect of peripheral endothelial dysfunction on clinical outcome following percutaneous coronary intervention (PCI) in patients with a CYP2C19 variant.

Methods: We enrolled 434 patients on DAPT following PCI. CYP2C19 genotype was examined, and we divided patients into two groups: carriers, who had at least one CYP2C19 LOF allele, and non-carriers. Peripheral endothelial dysfunction was examined using reactive hyperemia-peripheral arterial tonometry index (RHI), and we divided patients into low and high RHI. Thus, subjects were divided into four groups, and clinical events were followed up.

Results: A total of 55 patients had a cardiovascular event. Kaplan–Meier analysis demonstrated a significantly higher probability of cardiovascular events in carriers with low RHI (log-rank test: $p = 0.007$). Multivariate Cox proportional hazards analysis identified both CYP2C19 LOF allele possession (hazard ratio (HR): 1.94; 95% confidence interval (CI): 1.1–3.69; $p = 0.045$) and low RHI (HR: 2.15; 95% CI: 1.22–3.78; $p = 0.008$) as independent and significant predictors of future cardiovascular events.

Conclusions: CYP2C19 LOF allele carriers with peripheral endothelial dysfunction were significantly correlated with cardiovascular events. The additional evaluation of peripheral endothelial function along with CYP2C19 polymorphism might improve risk stratification after coronary stent implantation.

Introduction

Percutaneous coronary intervention (PCI) is performed in patients with coronary heart disease to improve symptoms and clinical prognosis. Coronary artery stents, particularly drug-eluting stents, are used to prevent abrupt closure of the stented artery, as well as to lower the need for repeat revascularization. Dual antiplatelet therapy (DAPT) is currently recommended for the prevention of adverse cardiovascular events in patients following coronary stenting [1–3]. Clopidogrel is the mainstay drug for DAPT; however, some patients do not achieve an adequate antiplatelet effect, and atherothrombotic events including stent thrombosis are not completely inhibited [4,5]. The antiplatelet efficacy of clopidogrel varies widely, and among

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several clinical characteristics, the genetic factors of cytochrome P450 (CYP) polymorphism correlate with diminished antiplatelet efficacy of clopidogrel and a high risk for adverse cardiovascular events following stent implantation [6–10]. The incidence of the CYP2C19 loss-of-function (LOF) genotype is higher in the Japanese population than in Caucasians [11], and we have demonstrated the relationship between CYP2C19 genotype and an increased risk of cardiovascular events in Japanese coronary heart disease patients following coronary stent implantation [6,12,13].

Reactive hyperemia-peripheral arterial tonometry (RH-PAT), which is used to measure the digital hyperemic response, is a noninvasive, automatic and less operator-dependent test that is clinically used to evaluate endothelial function [14,15]. It is reported that the RH-PAT index (RHI) predicted adverse cardiovascular events in patients without known coronary artery disease [16], and we have reported that the RHI was useful for identifying patients who were at high risk of ischemic heart disease [17,18].

We hypothesized that adding the evaluation of RHI as an assessment of peripheral endothelial function to CYP2C19 polymorphism would provide prognostic and predictive information of cardiovascular events in patients following coronary stent implantation.

Methods

The study complied with the Declaration of Helsinki regarding investigation in humans, was approved by our institutional review committee, and was conducted in accordance with the guidelines of an ethics committee. Written informed consent was obtained from all patients.

Study population

This was a prospective single-center study and a total of 600 consecutive patients who underwent PCI from January 2009 to November 2012 in our hospital were eligible. We excluded patients who were admitted for acute coronary syndrome; patients treated with thrombolytic agents, ticlopidine, sarpogrelate or cilostazol; and patients with deep vein thrombosis, atrial fibrillation, collagen disease, liver or renal dysfunction, and malignant diseases. Thus, a total of 434 patients were enrolled in this study. All patients underwent cardiac catheterization and PCI during hospitalization, and they received DAPT with maintenance doses of 100 mg/day of aspirin and 75 mg/day of clopidogrel after a loading dose of 300 mg of clopidogrel.

Smoking status was determined via an interview. Subjects were classified as having hypertension if they were receiving drug treatment for hypertension or if they had a systolic pressure of at least 140 mmHg or a diastolic pressure of at least 90 mmHg. Dyslipidemia was defined as low-density lipoprotein $\geq$140 mg/dl, high-density lipoprotein $<$40 mg/dl, or triglyceride $\geq$150 mg/dl; and diabetes as a 2-h glucose tolerance test finding of at least 200 mg/dl or a fasting glucose level of $\geq$126 mg/dl, hemoglobin A1c $\geq$6.5%, physician-diagnosed diabetes, and/or use of diabetic medication. Patients who had an ankle-brachial index value of $<$0.90 in either leg were categorized as having peripheral arterial disease. Chronic kidney disease was defined as estimated glomerular filtration rate $<$60 mL/min/1.73 m^2. Acute coronary syndrome was defined as either an acute myocardial infarction (ST-elevation myocardial infarction or non-ST-elevation myocardial infarction) or unstable angina pectoris according to the American College of Cardiology/American Heart Association guidelines [2,3].

CYP2C19 genotyping

Genomic DNA was extracted from whole blood using the DNA Extractor WB kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) following the modified protocol described by Richards et al. [19]. Polymerase chain reaction restriction fragment length polymorphism analysis for CYP2C19*2 (681G>A) and CYP2C19*3 (636G>A) was performed as described previously [20,21]. CYP2C19*2 and *3 are considered to account for >99% of alleles generating the null-activity enzyme in the Japanese population [20]. Therefore, the subjects were divided according to the CYP2C19 genotype into two groups: carriers: intermediate metabolizers (IM; *1/*2, *1/*3) and poor metabolizers (PM; *2/*2, *2/*3, *3/*3) carrying at least one CYP2C19 LOF allele, and non-carriers: extensive metabolizers (EM; CYP2C19*1/*1) not carrying a CYP2C19 LOF allele. In this study, we defined carriers as those who had at least one LOF allele and non-carriers as those who carried homozygous normal-function alleles.

Assessment of endothelial function by reactive hyperemia-peripheral arterial tonometry

Peripheral endothelial function was assessed by RH-PAT using the EndoPAT2000 system (Itamar Medical, Franklin, MA, USA). RH-PAT measurement is largely operator-independent, and a computerized algorithm with an online system automatically calculated the RH-PAT index (RHI); thus, there was minimal interoperator and intraoperator variability. The RH-PAT studies were performed as described previously [17]. We used a natural logarithmic transformation of the RH-PAT value to calculate the RHI: $\mathrm{RHI} = \mathrm{Ln}\{[\text{RH-PAT ratio}]1 \times [0.2266 \times \mathrm{Ln}(\text{baseline}) - 0.2]\}$ [17,22]. Previous studies demonstrated that RH-PAT technology has excellent reproducibility [23,24]. We used the median value of the RHI (0.55) to divide patients into low- and high-RHI groups.

Follow-up

After coronary stent implantation, patients were followed prospectively at outpatient clinics until October 2013 or until an endpoint occurred. We performed follow-up angiography 6–9 months after the procedure. We continued DAPT until the time of follow-up angiography in stable patients and until one year in acute coronary syndrome patients. We interrupted clopidogrel after that time, and partly continued DAPT in cases of PCI for left main trunk or complex lesions without a high risk of bleeding complications. Cardiovascular events were ascertained from a review of medical records and confirmed by direct contact with the patients, their families, and physicians. The endpoint was a composite of cardiovascular death, nonfatal myocardial infarction, ischemic stroke, unstable angina pectoris, coronary revascularization, and hospitalization due to heart failure. 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 [25]. The diagnosis of ischemic stroke was based on clinical and radiological evidence of stroke. Coronary revascularization was defined as emergent revascularization for unexpected hospitalization. In our institution, we performed PCI for coronary lesions with myocardial ischemia assessed by modalities such as electrocardiogram with exercise test, myocardial scintigraphy, or intraprocedural measurement of fractional flow reserve. We included ischemia-driven revascularization as the composite endpoint, and excluded revascularization therapy based only on angiographic data. For subjects who had more than 2 cardiovascular events, only the first event was considered in the analysis.