Thrombosis Research 132 (2013) 558–564

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Paraoxonase-1 activity affects the clopidogrel response in CYP2C19 loss-of-function carriers

Ryo Nishio, Toshiro Shinke *, Hiromasa Otake, Masayuki Nakagawa, Takumi Inoue, Hirotoshi Hariki, Tsuyoshi Osue, Yu Taniguchi, Masamichi Iwasaki, Noritoshi Hiranuma, Akihide Konishi, Hiroto Kinutani, Masaru Kuroda, Ken-ichi Hirata

Kobe University Graduate School of Medicine, Division of Cardiovascular Medicine, Department of Internal Medicine

ARTICLE INFO

Article history:
Received 10 May 2013
Received in revised form 26 July 2013
Accepted 9 September 2013
Available online 13 September 2013

Keywords:
Paraoxonase-1
CYP2C19
clopidogrel
OCT
VerifyNowP2Y12
intra-stent thrombus

ABSTRACT

Background: The impact of paraoxonase-1 (PON1) activity on the response to clopidogrel may differ in patients treated with drug-eluting stents (DES) in association with CYP2C19 loss-of-function (LOF) polymorphisms.
Methods: This study included 112 Japanese patients receiving clopidogrel (75 mg/day) and aspirin (100 mg/day) who underwent optical coherence tomography (OCT) examination 9 months after DES implantation. The CYP2C19 genotype was analyzed and LOF carriers (*1/*2, *1/*3, *2/*2, *3/*3, *2/*3) were identified. At the 9-month follow-up, platelet reactivity was determined by measuring the P2Y12 reactivity unit (PRU) using a VerifyNow P2Y12 assay, PON1 activity was evaluated and intra-stent thrombus was evaluated by OCT.
Results: Of the 112 Japanese patients, 75 were LOF carriers (67.0%). The patients were divided into tertiles according to the PON1 activity (tertile 1; <230 U/L, tertile 2; 230–283 U/L, tertile 3; >283 U/L). In the VerifyNowP2Y12 analysis, tertile 1 had a higher PRU than tertiles 2 and 3 in LOF carriers, and there was no difference among tertiles in non-carriers. The highest incidence of intra-stent thrombus was observed in tertile 1 followed by tertiles 2 and 3 in LOF carriers, whereas there was no such difference in non-carriers. Multivariate analysis revealed that LOF carriers and PON1 activity tertile 1 were independent predictors of intra-stent thrombus in all patients. In LOF carriers, tertile 1 was the only independent predictor for intra-stent thrombus.
Conclusion: Low PON1 activity is associated with a low response to clopidogrel and a high frequency of intra-stent thrombus only in LOF carriers.

Introduction

Clopidogrel is recommended as the first-line oral antiplatelet drug to prevent the recurrence of ischemic cardiovascular events after stent implantation [1]. A major limitation of clopidogrel therapy, however, is the large variability in the inter-individual response. Because clopidogrel is a pro-drug that requires a two-step enzymatic bioactivation to form the active metabolite that expresses its pharmacologic effects, and the hepatic cytochrome P450 (CYP) 2C19 is involved in both steps of the activation process, polymorphisms of genes encoding CYP2C19 are considered to be a major contributor to the variability in the inter-individual response to clopidogrel [2,3].

Paraoxonase-1 (PON1) has been emerged as another key factor for the second step of clopidogrel bioactivation. A recent study employing sophisticated metabolomic methods suggested a hydrolytic process involving PON1 as the rate-limiting enzyme without direct impact on platelet function. PON1enzyme gene variants and PON1 activity were reported as major determinants of clopidogrel responsiveness and a future thrombotic event after drug-eluting stent implantation [4]. Recent studies, however, failed to replicate the association between PON1 gene variants and clopidogrel responsiveness [5,6]. Therefore, the association between PON1 and clopidogrel efficacy remains controversial. In addition, the detailed interaction between the impact of PON1 activity on the clopidogrel bioactivation process or vessel healing within the stented segment and the presence of CYP2C19 gene variants remains unknown. Therefore, the aim of the present study was to clarify the effect of PON1 activity on clopidogrel responsiveness and vessel healing after drug-eluting stent (DES) implantation in association with CYP2C19 gene variants, using the VerifyNowP2Y12 assay (Accumetrics, San Diego, CA) and optical coherence tomography (OCT).

Abbreviations: PON1, paraoxonase-1; DES, drug-eluting stent; CYP, cytochromeP450; LOF, loss-of-function; OCT, optical coherence tomography; PRU, platelet reactivity unit; PCI, percutaneous coronary intervention; SES, sirolimus-eluting stent; PES, paclitaxel-eluting stent; EES, everolimus-eluting stent; TLR, target lesion revascularization; MI, myocardial infarction.

* Corresponding author at: Kobe University Graduate School of Medicine, Division of Cardiovascular and Respiratory Medicine, Department of Internal Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo, 650–0017, Japan. Tel.: +81 78 382 5846; fax: +81 78 382 5859.
E-mail address: shinke@med.kobe-u.ac.jp (T. Shinke).

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http://dx.doi.org/10.1016/j.thromres.2013.09.008

Methods

Patients

This study was approved by the ethics committee of Kobe University, and all enrolled study patients provided written informed consent to participate in the clinical trial and genetic study. Between April 2010 and April 2011, 198 patients at Kobe University Hospital underwent percutaneous coronary intervention (PCI) with a DES (sirolimus-eluting stent [SES]: Cypher™; Cordis Corp., Miami Lakes, FL; paclitaxel-eluting stent [PES]: TAXUS Liberte™; Boston Scientific Corporation, Natick, MA; everolimus-eluting stent [EES]: Promus™; Boston Scientific Corporation, Natick, MA; or Xience V™; Abbott Vascular, Santa Clara, CA). Of the 198 patients, 9 patients were lost to follow-up because they moved, 19 patients were excluded because of severe comorbidities unsuitable for repeat coronary angiogram (terminal stage cancer, end-stage renal dysfunction without dialysis, severe heart failure, severe allergy to the contrast agent, and dementia), and 14 patients did not provide consent. Thus, 156 patients agreed to undergo CYP2C19 polymorphism analysis as well as the planned follow-up angiography and OCT examination 9 months after PCI. Due to the recurrence of chest symptoms and evidence of myocardial ischemia on a stress test, 12 patients underwent premature repeat angiography. Among the 156 patients, patients with left main trunk disease and severe triple vessel disease ($n = 12$), severe tortuous lesions and severely calcified vessels ($n = 15$) were excluded to ensure the safety of the patients during the OCT procedure. Patients with vessels greater than 4.0 mm in diameter on angiography ($n = 5$) were excluded, because unsuitable for OCT imaging. Patients who discontinued clopidogrel administration due to the side effects ($n = 8$) were excluded. Thus, a total of 116 patients were enrolled into this study.

Patient characteristics, including age, sex, body mass index, and the presence of coronary risk factors were assessed. All patients treated with a DES received dual anti-platelet therapy. The loading dose of clopidogrel (300 mg) was administered before the procedure, followed by a maintenance dose of clopidogrel (75 mg/day) and aspirin (100 mg/day) for at least 1 year after the procedure. The compliance rate of dual antiplatelet therapy >80% was ensured at every one month when they visited the out-patient clinic.

Blood Sampling and Genotyping Methods

We obtained blood samples from the arterial sheath at the time of follow-up angiography. Genomic DNA was extracted from whole blood using the commercially available QIAamp™ DNA Blood Mini kit (QIAGEN N.V., Venlo, the Netherlands) according to the manufacturer's instructions. CYP2C19*2 (681G > A) or *3 (636G > A) polymorphisms were genotyped using TaqMan™ Drug Metabolism Genotyping Assays (Applied Biosystems, Foster City, CA) with the Applied Biosystem 7500 Real-Time PCR System. CYP2C19*2 and *3 are considered to account for more than 99% of alleles generating the null-activity enzyme protein in the Japanese population [7].

Platelet Function Testing

Platelet function was measured with the VerifyNow P2Y12 test just before follow-up angiography. This test measures ADP-induced platelet aggregation as an increase in light transmittance and utilizes a proprietary algorithm to report values in P2Y12 reaction units (PRU). A higher PRU result reflects greater P2Y12-mediated platelet reactivity [8].

Determination of PON1 Activity

At the time of the follow-up angiography, serum PON1 activity was measured by spectrophotometry, using paraoxon as a substrate. Briefly, for PON1 activity assays, the rate of the generation of p-nitrophenol was determined at 412 nm in 22-fold diluted serum. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 18,290 $M^{-1} \cdot cm^{-1}$ at 37 °C [9].

OCT Examination

To assess the relation between CYP2C19 polymorphisms and local vessel reaction after DES implantation, we performed OCT at the time of follow-up angiography. In this study, non-occlusive frequency-domain OCT with contrast injection was used. Briefly, a C7 Dragonfly ™ catheter (LightLab Imaging Inc., Westford, MA) was advanced to the distal end of the stented lesion over a 0.014-inch guide wire, followed by an infusion of contrast medium into the coronary artery from the guiding catheter at 3.5 ml/s, serving as a flush to clear the area of blood. The entire stented length was then imaged using an automatic pullback system moving at 20 mm/s.

OCT Analysis

Cross-sectional images were analyzed at 1-mm intervals. Neointimal thickness was measured from the center reflection of the stent strut to the vessel-lumen border (neointimal surface) for each stent strut [10]. Mean neointimal thickness was then calculated for each stent as the sum of these neointimal thicknesses divided by the total number of struts. A stent strut with a measured thickness of 0 μm was defined as an uncovered strut. Malapposed struts were defined as those with a maximum distance between the center reflection of the strut and the adjacent vessel surface of at least 170 μm for SES, at least 131 μm for PES, and at least 108 μm for EES [10]. The frequency of uncovered struts and malapposed struts was calculated as number of uncovered struts and malapposed struts divided by total number of struts. The stent eccentricity index and neointimal unevenness score were calculated as previously reported [11]. Intra-stent thrombus was defined as a mass protruding beyond the stent strut into the lumen with significant attenuation behind the mass [12]. A representative case of an intra-stent thrombus is shown in Fig. 1.

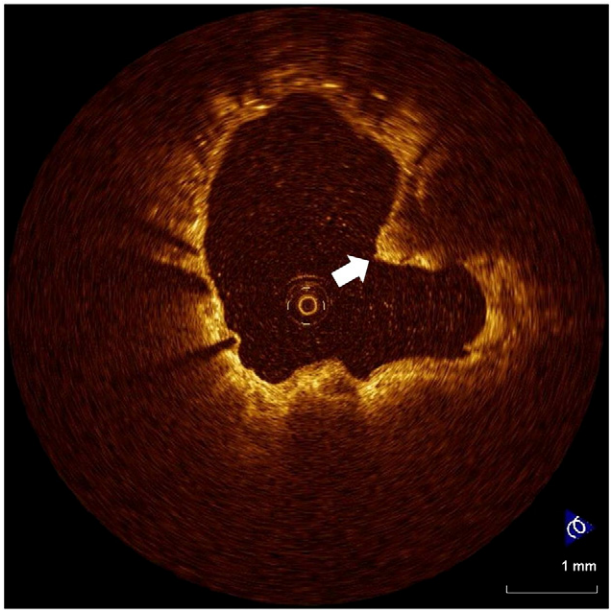

Fig. 1. Representative case of an intra-stent thrombus (white arrow), defined as a mass protruding beyond the stent strut into the lumen with remarkable attenuation behind the mass.