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Original Research Article

Role of genetic factors on the effect of additional loading doses and two maintenance doses used to overcome clopidogrel hyporesponsiveness

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ABSTRACT

Background and objective: Additional loading doses and higher maintenance doses (MDs) have been used to overcome hyporesponsiveness of clopidogrel. We aimed to investigate whether genetic polymorphisms of two cytochromes (CYP2C19 and CYP2C9) and ABCB1 modify effect of such dose-adjustment strategy.

Materials and methods: We enrolled 118 patients undergoing elective or acute percutaneous coronary intervention (PCI) with drug eluting stent (DES). Platelet reactivity index (PRI) was measured using the vasodilator-stimulated phosphoprotein (VASP) index and a cut-off value of ≥60% was defined as hyporesponsiveness. Polymorphism of two cytochromes (CYP2C19, CYP2C9) and gene ABCB1 were determined. In patients hyporesponsive to the initial LD the dose-adjustment was performed using up to 3 additional 600 mg LDs in order to achieve PRI <60%, and both 150 mg and 75 mg MD were tested at the follow-up.

Results: Patients with at least one CYP2C19*2 allele had higher baseline PRI after the initial LD (78.2 ± 13.1 vs. 65.3 ± 19.5, $P = 0.005$). The PRI reduction with additional LD was significantly smaller in carriers of the CYP2C19*2 (25.2 ± 15.6 vs. 35.5 ± 16.8, $P = 0.025$) and similar trend was observed with subsequent additional LDs. Both MDs were less effective in presence of CYP2C19*2. Target PRI was, however, more frequently achieved with higher MD even in presence of CYP2C19*2 (in 70.6% vs. 23.5% of hyporesponders, $P = 0.008$). No such differences were observed for other polymorphisms.

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Conclusions: In patients hyporesponsive to a routine clopidogrel doses the potency of additional LD and higher MD of clopidogrel is compromised by presence of CYP2C19*2 allele. The dose-adjustment strategy is not affected by ABCB1 C3435T or CYP2C9 genotypes.

1. Introduction

Dual antiplatelet therapy (DAPT) as a combination of aspirin and a $P2Y_{12}$-receptor antagonist reduces thrombotic complications in patients with acute coronary syndrome (ACS) or undergoing percutaneous coronary intervention (PCI), and it is a recommended treatment in current clinical guidelines [1]. For the last decade as a standard DAPT was the combination of aspirin and clopidogrel until newer generation more effective $P2Y_{12}$-receptor antagonists (prasugrel, ticagrelor) became available which provide more rapid, potent and reliable $P2Y_{12}$-receptor inhibition [1–3]. Although prasugrel and ticagrelor reduced the risk of cardiovascular death, myocardial infarction or stroke compared to clopidogrel in patients with ACS, the concerns of higher bleeding risk coupled with an increase in costs remain important shortcomings with the newer agents [4,5]. These considerations have encouraged the further investigation in a search for more personalized approach in each individual patient.

The pharmacodynamic response to clopidogrel varies among patients and standard doses of clopidogrel achieve suboptimal platelet inhibition. Hence, the "high on-treatment platelet reactivity" (HTPR) or hyporesponsiveness has been described in up to 50% of patients [2,6]. Numerous individual studies as well as several meta-analyses have demonstrated that HTPR is strongly associated with cardiovascular death, myocardial infarction and stent thrombosis (ST) in patients undergoing PCI [7].

Routine or platelet function testing-guided administration of higher or repeated clopidogrel loading doses (LDs) and higher maintenance doses (MDs) have failed to overcome hyporesponsiveness in a significant proportion of patients and yielded unsatisfactory long-term clinical results [8–15]. Genetic variants of CYP2C19 and ABCB1 genes have been associated with hyporesponsiveness and cardiovascular events among patients on treatment with clopidogrel [16]. Variations of these genes affect the rate of metabolism of clopidogrel that is pro-drug, and production of the active metabolite [16,17]. There are limited data if and how these polymorphisms affect the efficacy of tailored additional LDs and MDs used in hyporesponsive patients [18].

We aimed to investigate whether genetic polymorphisms of CYP2C19, ABCB1 and CYP2C9 modify effect of (i) additional 600 mg LDs of clopidogrel and (ii) higher MD (150 mg vs. 75 mg) in order to overcome hyporesponsiveness.

2. Materials and methods

In a prospective single-center study we included patients undergoing PCI with a drug eluting stent (DES) who received LD of clopidogrel according to the guidelines, namely, 300 mg or 600 mg for patients with scheduled or acute PCI, respectively [19–21]. The enrollment period was between September 2010 and December 2012. The following exclusion criteria were applied: expected noncompliance to therapy, congestive heart failure New York Heart Association functional class IV, bleeding or history of bleeding diathesis, platelet count $<100 \times 10^9$/L, oral anticoagulant therapy, chronic liver disease (cirrhosis, hepatitis) or serum bilirubin >2 mg/dL, hemorrhagic stroke or stroke of unspecified origin, malignancy or other concurrent severe illness with expected survival <1 year, contraindication to dual antiplatelet therapy as deemed by the treating physician. The protocol was approved by the local ethics committee and was according to the Declaration of Helsinki. Patients were included after two informed consents were obtained separately for each of two study components: treatment to clopidogrel and genetic investigation. Among initially included 118 patients only 94 patients fully adhered to the study design. One patient withdrew consent to the genetic analysis during the study therefore we report data on 93 patients. The remaining 24 patients were excluded during the study due to the following deviations from the protocol: incorrect use of clopidogrel doses ($n = 12$), treating physician changed clopidogrel to another antiplatelet drug ($n = 8$), patients refused a follow-up visit ($n = 4$). Minority of the patients ($n = 18$, 19.4%) underwent emergent or urgent PCI due to an acute coronary syndrome.

2.1. Blood samples

Blood samples for VASP phosphorylation analyses were drawn by atraumatic venipuncture of the antecubital vein. The first sample was taken after the PCI with DES on the second day after the routine LD. The subsequent samples were taken between 12 and 24 h after each additional LD, and at least 3 h after the last MD at the follow-up. Blood was collected into a vacutainer containing 3.8% trisodium citrate and filled to capacity. The vacutainer was inverted 3–5 times for gentle mixing and taken to the laboratory.

2.2. Platelet reactivity measurements

The VASP phosphorylation analysis was performed within 24 h of blood collection by an experienced investigator using Platelet VASP kits (PLT VASP/P2Y12, Biocytex, Marseille, France) according to the manufacturer's instructions [22]. A citrated blood sample was incubated with prostaglandin E_1 (PGE_1) and ADP 10 μmol/l for 10 min and fixed with paraformaldehyde, after which the platelets were permeabilized with a nonionic detergent. Analyses were performed on a Cytomics FC – 500 flow cytometer (Beckman Coulter, France), the platelet population was identified from its forward and side scatter distribution, and