Thrombosis Research 129 (2012) 616–622

Contents lists available at SciVerse ScienceDirect

Thrombosis Research

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

Regular Article

Influence of cytochrome 2C19 allelic variants on on-treatment platelet reactivity evaluated by five different platelet function tests

Thomas Gremmel [a], Christoph W. Kopp [a], Deddo Moertl [b], Daniela Seidinger [a], Renate Koppensteiner [a], Simon Panzer [c], Christine Mannhalter [d], Sabine Steiner [a,*]

[a] Division of Angiology, Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria
[b] Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria
[c] Clinical Department of Blood Group Serology and Transfusion Medicine, Division of Blood Group Serology, Medical University of Vienna, Vienna, Austria
[d] Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

ARTICLE INFO

Article history:
Received 1 July 2011
Received in revised form 7 October 2011
Accepted 10 November 2011
Available online 9 December 2011

Keywords:
Clopidogrel
CYP2C19
platelet function testing

ABSTRACT

Background: The antiplatelet effect of clopidogrel has been linked to cytochrome P450 2C19 (*CYP2C19*) carrier status. The presence of loss of function and gain of function variants were found to have a gene-dose effect on clopidogrel metabolism. However, genotyping is only one aspect of predicting response to clopidogrel and several platelet function tests are available to measure platelet response.

Patients and methods

We studied the influence of *CYP2C19* allelic variants on on-treatment platelet reactivity as assessed by light transmission aggregometry (LTA), the VerifyNow P2Y12 assay, the VASP assay, multiple electrode aggregometry (MEA), and the Impact-R in 288 patients after stenting for cardiovascular disease. Allelic variants of *CYP2C19* were determined with the Infiniti® CYP450 2C19+ assay and categorized into four metabolizer states (ultrarapid, extensive, intermediate, poor).

Results: Platelet reactivity increased linearly from ultrarapid to poor metabolizers using the VerifyNow P2Y12 assay ($P = 0.04$), the VASP assay ($P = 0.02$) and the Impact-R ($P = 0.04$). The proportion of patients with high on-treatment residual platelet reactivity (HRPR) identified by LTA, the VerifyNow P2Y12 assay and the VASP assay increased when the metabolizer status decreased, while no such relationship could be identified for results of MEA and Impact-R. The presence of loss of function variants (*2/*2, *2-8*/wt, *2/*17) was an independent predictor of HRPR in LTA and the VASP assay while it did not reach statistical significance in the VerifyNow P2Y12 assay, MEA, and the Impact-R.

Conclusion: Depending on the type of platelet function test differences in the association of on-treatment platelet reactivity with CYP2C19 carrier status are observed.

© 2011 Elsevier Ltd. All rights reserved.

Introduction

Clopidogrel is a cornerstone of antithrombotic therapy after percutaneous interventions with stent implantation. After metabolic activation of the prodrug through the cytochrome P450 (CYP) enzyme system, its metabolites bind and irreversibly block the platelet P2Y12 adenosine diphosphate (ADP) receptor [1]. Previous studies revealed a pronounced interindividual variability of clopidogrel metabolism [2], which was subsequently shown to influence the antiplatelet effects of clopidogrel treatment [3,4]. High on-treatment residual ADP-inducible platelet reactivity (HRPR) measured by ex vivo assays of platelet function has been associated with increased ischemic events compared to patients with adequate response to clopidogrel [3–9].

Recently, new ADP receptor inhibitors yielded promising results in large randomized clinical trials. In detail, prasugrel and ticagrelor were associated with a significant reduction of major adverse cardiovascular events compared to clopidogrel in patients with acute coronary syndromes [10,11]. Furthermore, both showed a faster,greater and more consistent inhibition of ADP-inducible platelet aggregation [12,13]. Consequently, these substances may become a therapeutic alternative in patients exhibiting HRPR during clopidogrel therapy.

Platelet reactivity upon clopidogrel treatment is influenced by a variety of factors including clinical conditions such as age and diabetes [14,15], co-medication such as calcium channel blockers and proton pump inhibitors (PPIs) [16,17], and the patient's genotype, in particular by several polymorphisms of *CYP2C19*. In detail, the *2

Abbreviations: CYP, cytochrome P450; ADP, adenosine diphosphate; HRPR, high on-treatment residual ADP-inducible platelet reactivity; LOF, loss-of-function; LTA, light transmission aggregometry; VASP, vasodilator-stimulated phosphoprotein; PRI, platelet reactivity index; PRU, P2Y12 Reaction Units; MEA, multiple electrode aggregometry; AU, aggregation units; SC, surface coverage.

* Corresponding author at: Division of Angiology, Department of Internal Medicine II, Medical University of Vienna, Waehringer Guertel 18–20, 1090 Vienna, Austria. Tel.: +43 1 40400 4670; fax: +43 1 40400 4665.
E-mail address: sabine.steiner-boeker@meduniwien.ac.at (S. Steiner).

0049-3848/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2011.11.019

allelic variant of *CYP2C19* was associated with an increased risk for adverse ischemic events indicating a loss-of-function (LOF) polymorphism, whereas carriers of the **17* allelic variant suffered significantly more bleeding events after stenting indicating a gain of function polymorphism [18–22]. Thus, a gene-dose effect was postulated for these variants [23].

The concept of using this information for individual risk assessment in patients on clopidogrel is appealing but it is still not clear whether to perform genotyping, testing of platelet reactivity, or both. Several platelet function tests are available, but are based on different principles and show only limited correlations among themselves [24,25]. Furthermore, data on the influence of *CYP2C19* polymorphisms on platelet reactivity are limited and none of the available studies used more than two platelet function tests.

Therefore, we studied the influence of *CYP2C19* allelic variants on on-treatment platelet reactivity as assessed by five different platelet function tests in patients treated with clopidogrel after intravascular stenting.

Materials and methods

Patients

In this observational study, 288 patients receiving aspirin and clopidogrel after intravascular stenting were recruited at the Medical University of Vienna, Division of Angiology, from January 2008 to November 2010.

Patients with therapy with vitamin K antagonists (warfarin, phenprocoumon, acenocoumarol), dipyridamol or nonsteroidal antiinflammatory drugs, a family or personal history of bleeding disorders, malignant paraproteinemias, myeloproliferative disorders or heparin-induced thrombocytopenia, severe hepatic failure, known qualitative defects in thrombocyte function, a major surgical procedure within one week before enrollment, a platelet count <100.000 or >450.000/μl and a hematocrit $<30\%$ were excluded from the study.

The study protocol was approved by the Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Blood sampling

Blood was drawn by clean venipuncture from an antecubital vein using a 21-gauge butterfly needle (0.8 x 19 mm; Greiner Bio-One, Kremsmünster, Austria) 24 hours after the percutaneous intervention. Thereby, blood samples were drawn after overnight fasting and without prior smoking for at least 12 hours. To avoid procedural deviations, all blood samples were taken by the same physician applying a light tourniquet, which was immediately released and the samples were mixed by gently inverting the tubes. The first 3 ml of blood were discarded to reduce procedurally induced platelet activation. The following aliquots of blood were drawn into a ethylenediamine-tetraacetic acid Vacuette tube (Greiner Bio-One) for DNA isolation, into a 3.8% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate 0.129 M/L) for light transmission aggregometry (LTA) and the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay, into a 3.2% sodium citrate Vacuette tube (Greiner Bio-One; 9 parts of whole blood, 1 part of sodium citrate 0.109 M/L) for the VerifyNow P2Y12 assay and into a Vacuette tube containing lithium heparin (18 IU/ ml) for the determinations by multiple electrode aggregometry (MEA) and the Impact-R. The time interval between blood sampling and testing was at least 1 hour and did not exceed 3 hours, all MEA measurements were performed between 1 and 2 hours after blood sampling. To avoid investigator-related variations of the results, each of the different tests was performed by just one corresponding operator, who was blinded to the results from the other operators.

Genotyping

Genomic DNA for genetic analysis was isolated from 400 μl EDTA blood with the MagNA Pure DNA-isolation system (Roche Diagnostics) according to the manufacturer's instructions. Allelic variants of CYP2C19 were determined with the Infiniti® CYP450 2C19+ assay (AutoGenomics, Carlsbad, CA, USA). The assay is designed to identify the following allelic variants (the nucleotide exchanges in the genes are shown in paranthesis): *CYP2C19*2* (19154 G>A), **3* (17948 G>A), **4* (1A>G), **5* (90033 C>T), **6* (12748 G>A), **7* (19294 T>A), **8* (12711 T>C), **9* (12784 G>A), **10* (19153 C>T), **17* (−806 C>T). Briefly, the procedure includes a multiplex PCR amplification of DNA (performed on an Eppendorf Mastercycler Gradient (Eppendorf North America, Inc., Westbury, N.Y., USA), incorporation of a fluorescent label using analyte specific primer extension (ASPE), and hybridization of the ASPE primers to a microarray followed by washing. All steps were performed exactly as recommended by the manufacturer. The microarrays were then subjected to signal detection and analysis on the Infiniti analyzer. Genotypes were determined blinded without knowledge of platelet aggregation values.

Light transmission aggregometry

LTA was performed on the APACT 4S Plus aggregometer (LABiTec, Ahrensburg, Germany) as previously described [24]. Platelet counts were not adjusted as the mean platelet count was 214 ± 3 G/L [26]. Aggregation was performed using ADP (10 μM; Rolf Greiner BioChemica, Flacht, Germany). Optical density changes were recorded photoelectrically for 10 minutes as platelets began to aggregate.

VerifyNow P2Y12 assay

The VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA) was performed as previously described [24]. With this assay, higher P2Y12 Reaction Units (PRU) reflect greater ADP-mediated platelet reactivity.

Vasodilator-stimulated phosphoprotein phosphorylation assay

The platelet reactivity index (PRI) was determined as previously described [24]. The extent of VASP phosphorylation was measured by geometric mean fluorescence intensity (MFI) values in the presence of PGE1 without (T1) or with ADP (T2). After subtraction of the negative isotypic control values from the corresponding fluorescence values, PRI (%) was calculated according to the following formula:

$$PRI\% = [T1(PGE1) - T2(PGE1 + ADP)/T1(PGE1)] \times 100$$

Multiple electrode platelet aggregometry

Whole blood impedance aggregometry was performed as previously described [24]. After addition of ADP (6.4 μM; Dynabyte, Munich, Germany), the adhesion of activated platelets to the electrodes led to an increase of impedance, which was detected for each sensor unit separately and transformed to aggregation units (AU) that were plotted against time.

Impact-R

The Impact-R ADP response test (DiaMed, Cressier, Switzerland) was performed as described [24]. Like in previous reports, platelet adhesion determined by examination of the percentage of total area

Download English Version:

https://daneshyari.com/en/article/6003313

Download Persian Version:

https://daneshyari.com/article/6003313

Daneshyari.com