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Aldona Kubica[1], Michał Kasprzak[2, 3], Karolina Obońska[2, 3], Marek Koziński[3], Eliano Pio Navarese[3], Grzegorz Grześk[2, 3], Katarzyna Linkowska[4], Katarzyna Stankowska[5], Tomasz Fabiszak[3], Joanna Boinska[5], Tomasz Grzybowski[4], Danuta Rość[5], Jacek Kubica[3]

[1]Department of Health Promotion,
[2]Department of Pharmacology and Therapy,
[3]Department of Cardiology and Internal Medicine,
[4]Department of Molecular and Forensic Genetics,
[5]Department of Pathophysiology,
Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Poland

Impact of CYP2C19 polymorphisms on antiplatelet efficacy of clopidogrel in patients after myocardial infarction

Corresponding author:

Aldona Kubica,
Technikow Street No 3,
86–801 Bydgoszcz, Poland
E-mail: aldona.kubica@gmail.com

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ABSTRACT

Aim. The aim of this study was to evaluate the complex effects of polymorphisms of the gene encoding the CYP2C19 enzyme on the antiplatelet efficacy of clopidogrel during follow-up visits.

Material and methods. This study was designed as a prospective, single-centre observational clinical trial with a one-year follow-up. Data from 178 patients on clopidogrel, taken during follow-up visits, was analysed.

Results. Patients were divided into three groups according to the expected metabolic activity of the CYP2C19 enzyme: 'superior metabolisers' (681 GG + 806 CT or TT), 'neutral metabolisers' (681 GG + 806 CC and 681 GA or AA + 806 CT or TT) and 'inferior metabolisers' (681 GA or AA + 806 CC). The antiplatelet effect of clopidogrel was strongest in 'superior metabolisers' and weakest in 'inferior metabolisers', as assessed by adenosine diphosphate (ADP)-induced platelet aggregation and the VASP assay. Comparison of results of ADP-induced platelet aggregation measurements in pairs showed a significant difference ($p = 0.03$) only between 'superior metabolisers' vs. 'inferior metabolisers'. With the VASP assay, significant differences between 'superior metabolisers' and 'neutral metabolisers' ($p = 0.013$), 'neutral metabolisers' and 'inferior metabolisers' ($p = 0.015$), and 'superior metabolisers' and 'inferior metabolisers' ($p = 0.00003$) were found. No impact on clinical outcome was present. A tendency for an increasing need for a clopidogrel-to-prasugrel switch was observed with a decrease in CYP2C19 metabolic activity.

Conclusion. Multiple polymorphisms of the gene encoding the CYP2C19 enzyme have a significant impact on the antiplatelet efficacy of clopidogrel during follow-up visits; however, their influence on clinical outcome needs further clarification.

Key words: antiplatelet therapy, clopidogrel, CYP2C19 polymorphism

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Introduction

According to current guidelines, myocardial infarction survivors require implementation of secondary cardiovascular prevention based on a long-term therapeutic schedule. One of the key elements of such a schedule, besides the modification of risk factors, is pharmacotherapy including antiplatelet agents: aspirin and P2Y12 receptor inhibitor. Despite the availability of new agents such as prasugrel or ticagrelor, clopidogrel remains the most widely used P2Y12 receptor inhibitor. Clopidogrel is a prodrug which requires a two-step oxidation in the liver involving cytochrome P450 enzymes to transform into active metabolite [1, 2]. Substantial variability of antiplatelet action is an important limitation of clopidogrel [3]. It has been shown that as many as 5–10% of patients do not respond to clopidogrel ('non-responders'), and approximately 25% are characterised by an incomplete response to the drug [4]. After excluding external causes of poor response to clopidogrel due to low patient adherence to medication or potential drug-drug interactions, genetic

factors should be taken into account [4, 5]. Genes encoding the cytochrome P450 enzymes are polymorphic and have a significant influence on the metabolism of clopidogrel [6, 7]. There are 25 known gene variants encoding the CYP2C19 enzyme [8]. Some of the alleles have a strong effect on the function of the enzyme [9–11]. The presence of two wild alleles (homozygote CYP2C19 *1/*1) implies normal CYP2C19 function. Mutations of CYP2C19 *2 and CYP2C19 *3 are the commonest variants causing loss of activity of the enzyme. Alleles CYP2C19 — *4 and *5 also cause inactivation of the enzyme, but they are very rare [12–16]. On the other hand, the CYP2C19 *17 mutation enhances the activity of the enzyme [17, 18]. Due to their prevalence and modifying influence on the enzymatic activity, two alleles: CYP2C19 –681 G>A and CYP2C19 –806C>T and –3402C>T, labelled as CYP2C19 *2 and CYP2C19 *17 respectively, have the greatest impact on the metabolism of clopidogrel. Almost all available knowledge regarding the impact of CYP2C19 metabolic activity on the antiplatelet effect of clopidogrel is based on acute-phase studies. Little is known about the complex effect of different polymorphisms on platelet aggregation in on-clopidogrel patients. Moreover, according to the contradictory results of several studies, an association between genetic variants of CYP2C19 and clinical outcome is dubious.

The aim of this study was to evaluate the complex effects of polymorphisms of the gene encoding the CYP2C19 enzyme on the antiplatelet efficacy of clopidogrel during follow-up visits.

Materials and methods

Study design and patient characteristics

This study was designed as a prospective, single-centre observational clinical trial with a one-year follow-up. The studied population comprised 191 consecutive patients treated with primary percutaneous coronary intervention for acute myocardial infarction, who gave informed written consent. Exclusion criteria were defined as follows: the need for prolonged use of heparin or fondaparinux, oral anticoagulant therapy, bleeding disorders (including thrombocytopenia $< 100 \times 103/\mu L$), anaemia (hemoglobin < 10.0 g/dL), active inflammation, heart failure in New York Heart Association class III and IV and life expectancy < 1 year. In-hospital management and discharge treatment recommendations strictly adhered to the European Society of Cardiology guidelines. Maintenance antiplatelet therapy consisted of clopidogrel 75 mg and aspirin 100 mg once a day. Follow-up visits were scheduled at three, six and nine months after discharge. During every visit, adenosine diphosphate (ADP)-induced platelet aggregation, platelet reactivity index (PRI) with the VASP assay and bleeding time were assessed. In cases of ADP-induced platelet aggregation > 41 U (borderline value of the highest tertile according to our previous studies) in patients who declared systematic intake of clopidogrel, the drug was switched to prasugrel.

The primary study end-point was defined as the necessity of a clopidogrel-to-prasugrel switch guided by ADP-induced platelet aggregation measurements. The secondary study end-point was a combined clinical end-point consisting of death, acute coronary syndrome and unscheduled cardiovascular hospitalization.

The study protocol was approved by the Ethical Committee of Nicolaus Copernicus University.

Platelet function assessment

ADP-induced platelet aggregation examination was performed using impedance aggregometry. Whole blood was tested with a Multiplate Analyser (Medical Cyclotron, Munich, Germany). Platelet aggregation rate, assessed as the area under the curve of the aggregation curve (AUC), was expressed in units of aggregation (U).

For the determination of vasodilator stimulated phosphoprotein phosphorylation in whole blood samples, a standardised reagent — Purified Mouse Anti-VASP (Becton, Dickinson & Co., Franklin Lakes, NJ, USA) and flow cytometer (Becton, Dickinson & Co., Franklin Lakes, NJ, USA) were used. Platelet reactivity with the VASP assay was expressed as PRI and determined based on the fluorescence of blood samples after the addition of the monoclonal antibody. PRI was defined as the mean fluorescence intensity (MFI) after incubation with PGE1 and ADP according to the following formula: $PRI = [(MFI_{(PGE1)} - MFI_{(PGE1+ADP)}/MFI_{(PGE1)}] \times 100$. The ratio is expressed as mean percentage platelet reactivity, inversely correlated with clopidogrel treatment efficiency.

CYP2C19 gene polymorphism assessment

Deoxyribonucleic acid was extracted from blood samples according to a standard organic procedure.

CYP2C19*17 (CYP2C19_-806_C>T, rs12248560) was genotyped with a commercially available validated drug metabolism genotyping assay (TaqMan Drug Metabolism Genotyping Assay C_469857_10, Applied Biosystems, Foster City, CA, USA) with the ABI Prism Sequence Detector 7000 (Applied Biosystems) in accordance with the manufacturer's instructions. CYP2C19*2 (CYP2C19_681_G>A; rs4244285) was genotyped with real-time allelic discrimination assay on an ABI Prism Sequence Detector 7000 (Applied Biosystems) as described previously [2].

The proper assessment of genotypes was evaluated in a random sequencing of PCR products using

a BigDye Terminator v. 3.1 sequencing kit and a 3130xl Genetic Analyser (Applied Biosystems). The compatibility between the results of real-time allelic discrimination and direct sequencing was confirmed. Genetic analysis was performed at the Department of Molecular and Forensic Genetics, CM UMK.

Statistical analysis

According to the Shapiro-Wilk test, the investigated continuous variables were non-normally distributed; therefore, they were reported as medians and interquartile ranges. For comparisons between two and three groups, the Mann-Whitney unpaired rank sum test and the Kruskal–Wallis one-way analysis of variance were used, respectively. Categorical variables were expressed as the number of patients presenting the given feature and the percentage of patients in the analysed group. Categorical variables were compared using the $\chi 2$ test with the Yates' correction if required. The Cochran–Armitage test was used to assess the presence of linear trend among categorical variables. Differences were considered significant at $p < 0.05$. The statistical analysis was carried out using the Statistica 10.0 package (StatSoft, Tulsa, OK, USA).

Results

A total of 191 patients were included into the study (Tab. 1). Thirteen of them withdrew from the study before the first follow-up visit. Data from 178 patients on clopidogrel, taken during follow-up visits (435 visits), was analysed.

Effects of single CYP2C19 polymorphisms

Due to the small number of patients being homozygous for CYP2C19*2 681 G > A, all carriers of the mutant alelle combined in one group consisting of homozygotes AA (n = 2) and heterozygotes GA (n = 33) were compared to GG homozygotes (n = 143). An analogous division was applied regarding the presence of the CYP2C19*17 -806 C> T mutant allele. TT homozygotes (n = 13) were combined with heterozygous CT patients (n = 72) and compared to CC homozygotes (n = 93). The analysis of ADP-induced platelet aggregation and PRI from follow-up visits in patients on the standard maintenance dose of clopidogrel showed an inhibitory effect of CYP2C19*2 (Tab. 2) and an escalating impact of CYP2C19*17 allele on the antiplatelet effect of clopidogrel (Tab. 3). No significant impact of both polymorphisms on the primary and secondary end-points was observed.

Complex effect of CYP2C19 polymorphisms

To allow for a comprehensive assessment of the impact of both these polymorphisms on the individual variation of clopidogrel antiplatelet action, patients were divided into three groups according to the expected metabolic activity of the CYP2C19 enzyme: 'superior metabolisers' (681 GG + 806 CT or TT), 'neutral metabolisers' (681 GG + 806 CC and 681 GA

Table 1. Characteristics of the studied population

Feature	Median (upper quartile — lower quartile) number (%) (n = 191)
Male	142 (74.3%)
Age [years]	60.0 (53.0–67.0)
Height [cm]	169.9 (164.0–176.0)
Body mass [kg]	80.0 (70.0–90.0)
Waist circumference [cm]	96.0 (89.0–103.5)
Body mass index > 25 kg/m^2	141 (73.8%)
Hypertension	106 (55.5%)
Diabetes	67 (35.1%)
Smokers	99 (51.8%)
LDL cholesterol ≥ 115 mg/dL	143 (74.9%)

Table 2. Results of ADP-induced platelet aggregation, PRI and bleeding time during follow-up visits with regard to the presence of CYP2C19*2 -681 G>A allele

CYP2C19*2 681 G>A	GG n = 143	GA or AA n = 35	p
ADP-induced platelet aggregation [U]	17.0 (10.0–27.0)	21.0 (16.0–33.0)	0.0005
PRI [%]	49.7 (34.2–63.0)	63.2 (46.2–74.9)	0.00003
Bleeding time [min]	4.0 (2.5–6.0)	3.5 (2.0–6.0)	ns
Primary study end-point	19 (13.3%)	7 (20.0%)	ns
Death	1 (0.7%)	0 (0.0%)	
ACS	8 (5.6%)	2 (5.7%)	
CV hospitalization	23 (16.1%)	7 (20.0%)	
Secondary study end-point	26 (18.2%)	7 (20.0%)	ns

Table 3. Results of ADP-induced platelet aggregation, PRI and bleeding time during follow-up visits with regard to the presence of CYP2C19*17 -806 C>T allele

CYP2C19*17 -806 C>T	CC n = 93	CT or TT n = 85	p
ADP-induced platelet aggregation [U]	19.0 (11.0–29.0)	17.0 (10.0–27.0)	ns
PRI [%]	54.7 (42.0–67.3)	48.6 (30.5–64.6)	0.006
Bleeding time [min]	4.0 (2.5–6.0)	3.5 (2.5–6.0)	ns
Primary study end-point	14 (15.1%)	12 (14.1%)	ns
Death	1 (1.1%)	0 (0.0%)	
ACS	6 (6.5%)	4 (4.7%)	
CV hospitalization	14 (15.1%)	16 (18.8%)	
Secondary study end-point	16 (17.2%)	17 (20.0%)	ns

Table 4. Results of ADP-induced platelet aggregation, PRI, bleeding time during follow-up visits and prevalence of study end-points with regard to the expected metabolic activity of CYP2C19 enzyme

	'Superior metabolisers' n = 71	'Neutral metabolisers' n = 86	'Inferior metabolisers' n = 21	p
ADP-induced platelet [U] aggregation	16.5 (9.0–26.0)	18.0 (11.0–29.0)	22.5 (15.5–32.5)	0.03
PRI [%]	47.9 (29.5–61.0)	53,7 (39.9–66.6)	63.3 (47.6–72.0)	< 0.0001
Bleeding time [min]	3.5 (2.5–5.5)	4.0 (3.0–6.0)	3.3 (2.0–5.5)	ns
Primary study end-point	9 (12.7%)	13 (15.1%)	4 (19.0%)	ns
Death	0 (0.0%)	1 (1.2%)	0 (0.0%)	
ACS	3 (4.2%)	6 (7.0%)	1 (4.8%)	
CV hospitalization	12 (16.9%)	15 (17.4%)	3 (14.3%)	
Secondary study end-point	13 (18.3%)	17 (19.8%)	3 (14.3%)	ns

or AA + 806 CT or TT), and 'inferior metabolisers' (681 GA or AA + 806 CC). The antiplatelet effect of clopidogrel was strongest in 'superior metabolisers' and weakest in 'inferior metabolisers', as assessed by ADP-induced platelet aggregation by PRI, but not by bleeding time (Tab. 4). Comparing the results of ADP-induced platelet aggregation measurements in pairs showed a significant difference (p = 0.03) only between extreme groups, i.e. 'superior metabolisers' vs. 'inferior metabolisers'. With PRI assessment, significant differences between 'superior metabolisers' and 'neutral metabolisers' (p = 0.013), 'neutral metabolisers' and 'inferior metabolisers' (p = 0.015), and 'superior metabolisers' and 'inferior metabolisers' (p = 0.00003) were found. Bleeding time did not differ between the groups.

During the observation period, the primary end-point occurred in 26 (14.6%), and the secondary end-point in 33 (18.5%) of the 178 patients.

Despite significant differences of on-clopidogrel platelet activity as assessed by ADP-induced platelet aggregation and PRI, no impact on clinical outcome was present. A tendency towards an increasing need for a clopidogrel-to-prasugrel switch was observed with a decrease in CYP2C19 metabolic activity (Fig. 1).

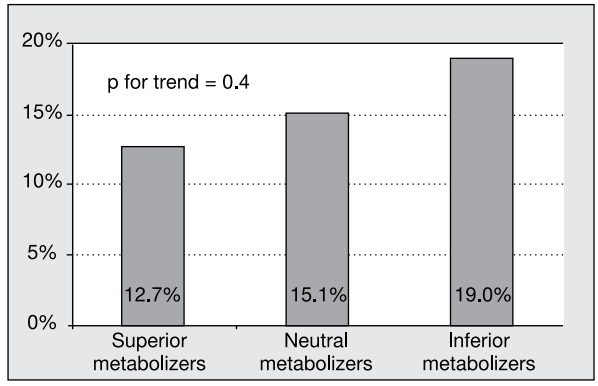

Figure 1. Proportion of patients requiring clopidogrel-to-prasugrel switch according to CYP2C19 metabolic status

Discussion

CYP2C19 and clopidogrel pharmacodynamics

Combinations of different CYP2C19 alleles cause varied effects on the activity of this enzyme and, consequently, on the metabolism of clopidogrel [19]. The prevalence of alleles is ethnically variable. A study published by Sibbing

et al. [20] showed that in a German population, 73% were CYP2C19*1/*1 homozygotes, 25% were heterozygotes with CYP2C19*2 as one of two alleles, and only 2% were CYP2C19*2/*2 homozygotes. Similar prevalences have been found in French and Polish populations [9, 21]. Due to the relatively rare occurrence of CYP2C19*2/*2 genotype, the analysis in clinical trials is usually based on a comparison of homozygotes CYP2C19*1/*1 with carriers of at least one CYP2C19*2 loss-of-function mutant allele (CYP2C19*1/*2 or CYP2C19*2/*2). Carriers of CYP2C19*2 are more common in populations of non-European ancestry; they account for about 40% of patients of African ancestry and over 55% of East Asian populations [5, 22]. The CYP2C19*17 allele is relatively common, being observed in 43% of Poles, 41% of Germans, 20% of French, 18% of Swedes and Ethiopians, and only 4% of the Chinese [17, 18, 21].

Hulot et al. [13] found a significant reduction of ADP-induced platelet aggregation in CYP2C19*1/*1 homozygotes, while in carriers of CYP2C19*2 allele the response to clopidogrel was much weaker in healthy subjects. Mega et al. [5] revealed significantly lower concentrations of clopidogrel active metabolite in CYP2C19*2 carriers compared to non-carriers. This was in line with pharmacodynamic findings: the maximum induced platelet aggregation was about 25% lower among carriers of the loss-of-function allele. These observations were confirmed in a group of 797 consecutive patients who underwent coronary angioplasty [8]. Carriers of the CYP2C19*2 allele were characterised by higher residual platelet aggregation, both after the loading dose (600 mg) and on the maintenance dose (75 mg) of clopidogrel. This phenomenon was also confirmed in our study by PRI and ADP-induced platelet aggregation measurements performed during follow-up.

Frere et al. [10] studied the effect of CYP2C19*17 allele on the metabolism of clopidogrel in patients with acute coronary syndrome. The loading dose of clopidogrel (600 mg) was associated with a significantly greater reduction in PRI in carriers of CYP2C19*17 compared to non-carriers. In another study, clopidogrel-treated patients undergoing percutaneous coronary intervention, both heterozygous CYP2C19*1/*17 and those who were CYP2C19*17/*17 homozygotes, were characterised by significantly lower ADP-induced platelet aggregation than CYP2C19*1/*1 homozygotes [23]. In this study, we proved enhancement of clopidogrel metabolism according to follow-up PRI measurements.

Comprehensive assessment of both CYP2C19*2 and CYP2C19*17 genetic variants may better reflect the individual variation of clopidogrel's antiplatelet action. We defined three groups according to the expected metabolic activity of the CYP2C19 enzyme. As expected, the attenuation of platelet aggregation was highest in 'superior metabolisers' and lowest in 'inferior metabolisers', as assessed by ADP-induced platelet aggregation by PRI.

CYP2C19 and clinical outcome

Pharmacodynamic and pharmacokinetic findings were consistent with the results of some recently published clinical studies [14, 24]. Collet et al. [9] found an increased incidence of coronary events in CP2C19*2 allele carriers compared to CYP2C19*1/*1 homozygotes. In addition, the carriage of the loss-of-function allele was an independent risk factor for the composite end-point of death, myocardial infarction, and urgent revascularisation [9]. In a study published by Sibbing et al. [20], the carriage of CYP2C19*2 allele was associated with a higher risk of myocardial infarction and ischaemic stroke ($p = 0.001$) in patients treated with clopidogrel due to stent implantation. The presence of this allele was an independent risk factor of stent thrombosis [20]. In the genetic substudy of the TRITON-TIMI 38 trial, the risks of the composite end-point (death from cardiovascular causes, myocardial infarction, stroke), and of stent thrombosis, were significantly higher among carriers of CYP2C19*2 allele compared to non-carriers [5]. In a registry published by Tang et al. [22], the risk of ischaemic events increased with the incidence of CYP2C19 loss-of-function alleles in 670 patients after percutaneous coronary intervention. On the other hand, Tello-Montoliu et al. [25] revealed that the VASP phosphorylation measurement detected significant differences in on-clopidogrel platelet reactivity between the wild-type subjects and the CYP2C19*2 or *17 allele carriers, although no significant difference in the occurrence of adverse events at six-month follow-up was found. Similarly, the carriage of CYP2C19 loss-of-function alleles as well as gain-of-function alleles did not influence the rate of ischaemic events in a population of 4,819 stable patients with stable coronary artery disease in the CHARISMA genetics study [26]. A meta-analysis published by Zabalza et al. [24] also showed that patients who carried a loss-of-function allele did not present with an increased risk of a cardiovascular event, except for stent thrombosis. However, the gain-of-function allele was associated with a lower risk of cardiovascular events and a higher risk of major bleeding [24]. Similarly, data from six clinical studies has demonstrated that carriers of the CYP2C19*17 variant had a marked protection against recurrent cardiovascular events in patients with coronary artery disease compared to non-carriers [27]. Both in separate analyses and in comprehensive evaluation of CYP2C19*2 and CYP2C19*17 carriage, we have not found significant impacts on the primary nor the secondary end-point, although the proportion of patients requiring a clopidogrel-to-prasugrel switch well reflected the results of ADP-induced platelet aggregation measurements and PRI. The prevalence of the combined clinical end-point did not follow the results of platelet function assessment. Inconsistency between platelet aggregation and the incidence of the secondary end-point

can be at least partially related to a higher proportion of drug switch for the stronger P2Y12 inhibitor in patients with a weak effect of clopidogrel.

Limitations of the study

The main limitation of this study was uncertainty regarding patients' adherence to medication during follow-up. Blood samples for the evaluation of platelet function were taken only from patients declaring systematic intake of clopidogrel; however, this was not objectively proved.

Conclusions

Multiple polymorphisms of the gene encoding the CYP2C19 enzyme have a significant impact on the antiplatelet efficacy of clopidogrel during follow-up visits, but their influence on clinical outcome needs further clarification.

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