Influence of C3435T multidrug resistance gene-1 (MDR-1) polymorphism on platelet reactivity and prognosis in patients with acute coronary syndromes

Mateusz Śpiewak[1,2], Łukasz A. Małek[1], Grażyna Kostrzewa[3], Bartłomiej Kisiel[4], Agnieszka Serafin[2], Krzysztof J. Filipiak[2], Rafał Płoski[3], Grzegorz Opolski[2]

[1] 1st Department of Coronary Artery Disease, Institute of Cardiology, Warsaw, Poland

[2] 1st Department of Cardiology, Medical University, Warsaw, Poland

[3] Department of Medical Genetics, Medical University, Warsaw, Poland

[4] Department of Internal Diseases and Rheumatology, Military Institute of the Health Services, Warsaw, Poland

Abstract

Background: Genetic C3435T polymorphism of the multidrug resistance gene-1 (MDR-1) limits oral bioavailability of clopidogrel and influences prognosis in patients with myocardial infarction.

Aim: To assess the effects of C3435T polymorphism on platelet reactivity and prognosis in patients with acute coronary syndromes treated with percutaneous coronary intervention with stenting.

Methods: Ninety-eight patients were divided into subgroups according to closure time (CT) measured with the Platelet Function Analyzer-100 by means of collagen/adenosine diphosphate (CADP) and collagen/epinephrine (CEPI) cartridges. Patients with CADP-CT < 130 s and patients with CEPI-CT ≤ 193 s were defined as non-full responders to antiplatelet therapy. Patients with coexisting polymorphism of P2Y12 and CYP2C19 genes were excluded from the analysis. The primary outcome was the composite of death, non-fatal myocardial infarction and non-fatal stroke.

Results: Patients carrying the homozygous TT genotype were more likely to have an impaired response to antiplatelet therapy in the test with ADP in comparison to carriers of homozygous CC genotype (OR 5.23; 95% CI 1.34-20.45; p = 0.017). For CT heterozygotes in comparison to CC genotype a weak trend toward non-full response to antiplatelet therapy in the CADP test was observed (OR 2.71; 95% CI 0.80-9.14; p = 0.11). No relationship between MDR-1 C3435T polymorphism and response in CEPI test was observed either for TT homozygotes or for heterozygotes (p = 0.57 and p = 0.55, respectively). During a mean 1.7 years of follow-up no significant difference in the risk of the primary end point was observed.

Conclusions: The C3435T polymorphism of the MDR-1 gene influences ADP dependent platelet reactivity in patients with acute coronary syndrome but does not affect mid-term prognosis in this population.

Key words: antiplatelet drugs, acute coronary syndromes, multidrug resistance polymorphism, response variability

Kardiol Pol 2009; 67: 827-834

Introduction

Antiplatelet drugs are recommended as one of the main medications used in patients across the whole spectrum of acute coronary syndromes (ACS) [1, 2]. However, several studies have demonstrated that a substantial group of patients has an impaired platelet response to clopidogrel and/or aspirin measured in various tests of platelet reactivity [3-5]. Poor response to antiplatelet therapy and therefore high platelet reactivity are associated with a higher risk of cardiovascular events [4-12]. Lack of adequate platelet inhibition might be associated with many possible factors including patients' non-compliance, drug interactions, alternative mechanisms of platelet activation, but also polymorphism of genes which play a role in clopidogrel function [3, 13].

Several gene polymorphisms of different steps of clopidogrel action influencing drug biotransformation to its active metabolite by hepatic cytochrome P450 or platelet response to adenosine diphosphate (ADP) have been

Address for correspondence:

Mateusz Śpiewak MD, I Klinika Choroby Wieńcowej, Instytut Kardiologii, ul. Alpejska 42, 04-628 Warszawa, tel.: +48 22 343 42 72, e-mail: mspiewak@ikard.pl

Received: 24 January 2009. **Accepted:** 07 May 2009.

The study was supported by the scientific grants 1WR/NM1/05, 1WR/NM2/05 and 1WY/W1/06 from the Medical University of Warsaw, Poland.

extensively studied in the last years [13-22]. Recent data suggest that the main determinant of adequate clopidogrel function is related to the concentration of its active metabolite in plasma instead of platelet receptor defects [23]. Apart from the activating hepatic biotransformation, one of the determinants of plasma concentration of the drug may be its intestinal absorption. It has been shown that multidrug resistance gene-1 (MDR-1) polymorphism influences oral bioavailability of clopidogrel and influences prognosis of patients with myocardial infarction [13, 24]. The MDR-1 (also called ABCB1) gene encodes P-glycoprotein responsible for cellular efflux of many medications [25]. Patients with two variant alleles of MDR-1 (TT genotype) had lower plasma concentrations of clopidogrel and its active metabolite when compared with carriers of the CT and CC genotypes [24]. There are no studies evaluating the influence of MDR-1 C3435T polymorphism on platelet activation and outcomes in patients with the whole spectrum of ACS. Therefore, we decided to determine the role of MDR-1 C3435T polymorphism on platelets' response to antiplatelet therapy and clinical events in ACS survivors treated with percutaneous coronary intervention (PCI) with stent implantation.

Methods

Study population

Hundred five patients with ACS undergoing PCI with bare metal stent were screened. Seven patients with coexisting polymorphism of P2Y12 (i-T744C, genotype CC or CT) and CYP2C19 (G681A, genotype AA or GA) genes, shown to have significantly affected platelet response to clopidogrel treatment, were excluded from the present analysis [26]. All patients were treated with a loading dose of aspirin (300 mg) and clopidogrel (300 mg or 600 mg) followed by 75 mg of aspirin and 75 mg of clopidogrel daily according to the current guidelines [27]. Exclusion criteria were: concomitant glycoprotein IIb/IIIa inhibitor administration, recent or chronic clopidogrel treatment, active neoplasm or a history of neoplasm, severe renal insufficiency (plasma creatinine > 4 mg/dl) or severe hepatic insufficiency, haemorrhagic diathesis, haematocrit < 35% or > 50%, total platelet count < 150 000 µl or > 500 000 µl and alcohol abuse.

Written informed consent was obtained from each patient. The study was approved by the local ethics committee and conformed with the principles outlined in the Declaration of Helsinki.

Platelet function analysis

Measurements of platelet activity were performed with a point-of-care device [Platelet Function Analyzer (PFA)-100, Dade Behring, Germany]. Tubes with 3.2% (0.105 M) buffered sodium citrate were used for blood collection. PFA-100 analysis was performed within 2 h of sampling. The mechanism of action of PFA-100 was described previously [26]. In brief, in PFA-100 two cartridges with collagen/adenosine diphosphate (CADP) and collagen /epinephrine (CEPI) as platelet activators are used. The system measures time to blood flow termination through a 150 µm central aperture under high shear stress. The result is given as closure time (CT). After 300 s the process automatically terminates, and this value reveals the maximal platelet inhibition. Simultaneous measurements of CADP closure time (CADP-CT) and CEPI closure time (CEPI-CT) according to manufacturer's instructions were performed. The cut-off values pre-specified by previous studies for impaired inhibition of platelets were CADP-CT < 130 s (for ADP-dependent activation) and CEPI-CT ≤ 193 s (for epinephrine-dependent activation) [11, 29-31].

Genetic analysis

Blood samples (10 ml) for genetic tests were taken from each patient. Genotyping was performed using allele-specific polymerase chain reaction (PCR) with primers designed with the tetra-primer amplification refractory mutation system (available at: http://cedar.genetics.soton.ac.uk/public_html/primer1.html) [32]. PCR was performed in incubation buffer (50 mM KCl, 10 mM Tris-HCl, pH = 8) with 5 mM $MgCl_2$, 20 mM of each dNTP, 180 ng of template DNA, and 1.6 U Taq polymerase (Fermetas, Vilnius, Lithuania) in the final reaction volume of 10 µl with 2 pM of each outer primer (forward outer primer 5'-TAGGCCAGAGAGGCTGCCACATGCTCCC-3' and reverse outer primer 5'-TCGTGTCCCAGGAGCCCATCCTGTTTGA-3') and 10 pM of each allele-specific (inner) primer: forward inner primer 5'-TATGTTGGCCTCCTTTGCTGCCCTCCCA-3' and reverse inner primer 5' CAGCCGGGTGGTGTCACAGGAA -GAGCTC-3' (available at: http://genewindow.nci.nih.gov/LocusRequest?locus=NT_007933:12363224-12580840&q=rs1045642). Reactions were run in the 2720 Thermal Cycler (Applied Biosystems) under the following conditions: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 65.5°C for 55 s, and extension at 72°C for 1 min with a final extension of 7 min at 72°C. PCR products were resolved on non-denaturing 3.0% agarose gels containing ethidium bromide and visualised under ultraviolet illumination. The amplification products obtained from the DNA of individuals heterozygous (CT) for the polymorphism showed three bands: 201 bp, 137 bp and 120 bp. In CC homozygotes upon electrophoresis of the amplified fragment, two bands, 201bp and 137bp, were observed. In TT homozygotes, also two bands (201 bp and 120 bp) were seen. All genetic analyses were repeated twice for each patient and were performed by an operator blinded to platelet function results.

Outcome measures

The end point of the study was the composite of death, non-fatal myocardial infarction and non-fatal stroke. After

12 months from hospital discharge the status of each patient was checked by telephone contact or by letter when telephone contact was impossible. Next evaluations were performed every six months up to 24 months.

Statistical analysis

All results for continuous variables are expressed as means ± standard deviation and skewed variables as the median with interquartile range (IQR). For analysis of relations between categorical variables we used the chi-square test, χ^2 test for trend or Fisher's exact test when appropriate. T-test or Wilcoxon rank sum test for unpaired samples was used to compare any continuous variables with a normal or non-normal distribution, respectively. To test for Hardy-Weinberg equilibrium for each polymorphism, the expected genotype numbers were calculated from the allele frequencies, and deviation from the observed genotype numbers was determined using the chi-square test. In order to test for the effect of each genotype and their interaction the odds ratios (ORs) and their 95% confidence intervals (95% CI) were computed by unconditional logistic regression analysis using the maximum likelihood method. All tests were 2-sided. A p values less than 0.05 were considered to indicate statistical significance. All statistical analyses were performed with SAS software version 8e (SAS Institute Inc, Cary, NY, USA).

Results

Baseline characteristics

The baseline characteristics of the study group and the subgroups divided according to CADP-CT and CEPI-CT are shown in Table I. There were no significant differences in relation to any of the analysed parameters between groups except a trend toward more frequent history of stroke in the group with excessive ADP-dependent platelet reactivity as compared with patients with full platelet inhibition after the CADP test (10.0 vs. 0%;

Table I. Baseline characteristics of the studied population

Parameter	Total group n = 98	Non-full inhibition in CADP test n = 30	Full inhibition in CADP test n = 68	Non-full inhibition in CEPI test n = 21	Full inhibition in CEPI test n = 77
Male gender, n (%)	69 (70.4)	22 (73.3)	47 (69.1)	17 (80.9)	52 (67.5)
Mean age (SD)	60.3 (11.5)	62.2 (11.1)	59.5 (11.6)	59.8 (9.0)	60.6 (12.1)
Loading dose of clopidogrel					
300 mg, n (%)	29 (29.6)	7 (23.3)	22 (32.3)	3 (14.3)	26 (33.8)
600 mg, n (%)	69 (70.4)	23 (76.7)	46 (67.7)	18 (85.7)	51 (66.2)
Median day of sampling (IQR)	6 (6-6)	6 (5-7)	6 (6-6)	6 (5-7)	6 (6-6)
Previous MI, n (%)	16 (16.3)	6 (20.0)	10 (14.7)	5 (23.8)	11 (14.2)
Prior PCI or CABG, n (%)	9 (9.2)	3 (10.0)	6 (8.8)	3 (14.3)	6 (11.7)
PAD, n (%)	8 (8.2)	3 (10.0)	5 (7.3)	1 (4.8)	7 (9.1)
Prior stroke, n (%)	3 (3.0)	3 (10.0)*	0 (0.0)*	2 (9.5)	1 (1.3)
Diabetes mellitus, n (%)	17 (17.3)	5 (16.6)	12 (17.6)	6 (28.6)	11 (14.2)
Dyslipidaemia, n (%)	35 (35.7)	8 (26.7)	27 (39.7)	10 (47.6)	25 (32.5)
Hypertension, n (%)	52 (53.1)	13 (43.3)	39 (57.3)	13 (61.9)	39 (50.6)
Current smoker, n (%)	43 (43.9)	14 (46.7)	29 (42.6)	11 (52.4)	32 (41.6)
STEMI, n (%)	80 (81.6)	24 (80.0)	56 (82.3)	15 (71.4)	65 (84.4)
MVD, n (%)	17 (17.3)	4 (13.3)	13 (19.1)	2 (9.5)	15 (19.5)
Platelet count × 10^9/l (SD)	225.5 (61.3)	218.6 (58.1)	227.8 (62.9)	221.0 (52.3)	225.7 (64.2)
Haematocrit (SD)	39.4 (4.7)	39.4 (4.3)	39.4 (4.9)	39.8 (3.7)	39.3 (5.0)
Pharmacotherapy, n (%)					
aspirin	98 (100)	30 (100)	68 (100)	21 (100)	77 (100)
clopidogrel	98 (100)	30 (100)	68 (100)	21 (100)	77 (100)
heparin (UFH/LMWH)	98 (100)	30 (100)	68 (100)	21 (100)	77 (100)
beta-blockers	98 (100)	30 (100)	68 (100)	21 (100)	77 (100)
ACE-I	97 (99.0)	30 (100)	67 (98.5)	21 (100)	76 (98.7)
statin	98 (100)	30 (100)	68 (100)	21 (100)	77 (100)

Abbreviations: CADP-CT – collagen/adenosine diphosphate closure time, CEPI-CT – collagen/epinephrine closure time, SD – standard deviation, IQR – interquartile range, MI – myocardial infarction, MVD – multivessel disease, PCI – percutaneous coronary intervention, CABG – coronary artery bypass graft, PAD – peripheral artery disease, STEMI – ST-elevation myocardial infarction, UFH – unfractionated heparin, LMWH – low molecular weight heparin
** p = 0.057*

p = 0.057). The genetic analysis yielded the same results in both series. The genotype distribution in the whole group was as follows: 21 TT homozygotes (21.4%), 50 CT heterozygotes (51.0%) and 27 CC homozygotes (27.6%). The genotype frequencies were consistent with Hardy-Weinberg predictions and similar to those reported in the Polish population (Table II) [33-38].

Influence of C3435T polymorphism on platelet function

There was a significant difference in genotype distribution between groups with full and non-full platelet inhibition in CADP (Table III A). Patients with platelet overactivity after ADP stimulation had a higher frequency of TT genotype when compared with patients with full response to antiplatelet therapy (33.3 vs. 13.2%, p = 0.01). Patients carrying the homozygous TT genotype were more likely to have excessive platelet activity despite antiplatelet treatment measured in the test with collagen/ADP cartridges in comparison to carriers of the homozygous CC genotype (OR = 5.23; 95% CI 1.34-20.45; p = 0.017). For CT heterozygotes a weak trend toward ADP-dependent platelet overactivity was observed (OR = 2.71; 95% CI 0.80-9.14; p = 0.11) in comparison to CC genotype. No differences in genotype distribution were observed in relation to platelet response to epinephrine and collagen stimulation (Table III B; p = 0.54). Patients carrying the homozygous TT genotype or heterozygous CT genotype had similar odds to have non-full platelet inhibition as measured using CEPI-CT (OR = 0.67; 95% CI 0.17-2.69; p = 0.57 and OR 0.71; 95% CI 0.24-2.16; p = 0.55, respectively) in comparison to CC genotype carriers. The frequency of non-full platelet inhibition after ADP stimulation according to genotype was as follows: 47.6% in patients with TT genotype, 32.0% in patients with CT genotype and 14.8% in CC homozygotes (p = 0.01; Figure 1 A). For epinephrine-dependent platelet activity despite the antiplatelet therapy these results were: 19.0%, 20.0% and 25.9%, respectively (p = 0.54; Figure 1 B).

Clinical outcome

The mean duration of follow-up was 1.7 years. None of the 98 patients enrolled in the study was lost to follow-up. The end point occurred in 10 patients (10.2%). There were 3 cardiovascular deaths and 7 cases of non-fatal myocardial infarction. There were no cases of non-fatal stroke. Among patients with TT genotype 3

Table II. Genotype and allele distribution in the study group with comparison to healthy subjects in other studies in the Polish population (no significant differences were observed)

	Allele frequencies		Genotype frequencies		
	C	T	CC	CT	TT
This study, n = 98	0.53	0.47	0.28	0.51	0.21
Jamroziak et al., 2002 [33], n = 122	0.62	0.38	0.42	0.41	0.17
Jamroziak et al., 2004 [34], n = 175	0.60	0.40	0.40	0.41	0.19
Tan et al., 2004, [35] n = 139	0.48	0.52	0.22	0.52	0.26
Kurzawski et al., 2005 [36], n = 188	0.51	0.49	0.26	0.50	0.24
Kurzawski et al., 2006 [37], n = 204	0.47	0.53	0.22	0.51	0.27
Jamroziak et al., 2009 [38], n = 96	0.54	0.46	0.24	0.59	0.17

Table III. Genotype frequencies according to platelet activity in response to ADP (**A**) and epinephrine (**B**) stimulation

A				
Phenotype	Total no.	MDR-1 3435 genotype		
		CC, n (%)	CT, n (%)	TT, n (%)
Non-full inhibition of ADP-dependent pathway	30	4 (13.3)	16 (53.3)	10 (33.3)
Full inhibition of ADP-dependent pathway	68	23 (33.8)	34 (50.0)	11 (16.2)
$\chi^2 = 6.07$, p = 0.01				
B				
Phenotype	Total no.	MDR-1 3435 genotype		
		CC, n (%)	CT, n (%)	TT, n (%)
Non-full inhibition of epinephrine-dependent pathway	21	7 (33.3)	10 (47.6)	4 (19.0)
Full inhibition of epinephrine-dependent pathway	77	20 (26.0)	40 (51.9)	17 (22.1)
$\chi^2 = 0.37$, p = 0.54				

(14.3%) patients died or suffered from non-fatal myocardial infarction. In patients with CT genotype the primary outcome occurred in 6 (12.0%) patients and in CC homozygotes in 1 (3.7%) patient. These differences did not reach statistical significance. Clinical outcome in the studied population according to MDR-1 genotype is presented in Table IV.

Discussion

We have demonstrated that C3435T polymorphism of the MDR-1 gene may influence platelet function as assessed by means of CADP-CT with PFA-100. The most probable mechanism behind this association may be related to decreased intestinal absorption of clopidogrel in patients with TT genotype as compared to carriers of CC genotype.

Importantly, we did not reveal any relationship between C3435T MDR-1 polymorphism and platelet function as measured with CEPI-CT.

Several studies performed to determine the influence of C3435T polymorphism on function of P-glycoprotein and its substrates have provided conflicting results, indicating that the same genotype can be associated with increased, decreased or non-influenced plasma levels of substrates of P-glycoprotein, depending on the studied population and analysed substrate [39-43]. To our knowledge only one study (in a German population) has assessed the linkage between C3435T polymorphism and plasma levels of clopidogrel and its active metabolite, indicating that TT genotype leads to diminished plasma concentrations of clopidogrel and its active metabolite [24]. In a recent study by Simon et al. it was demonstrated that 3435TT genotype was associated with worse clinical outcome in patients with myocardial infarction receiving clopidogrel but not in patients without clopidogrel therapy [13]. Moreover, the interaction between clinical events and MDR-1 genotype was present only in the whole studied population (patients treated with PCI as well as patients treated with thrombolysis, conservative therapy or coronary artery bypass grafting) but not in patients who undergo PCI.

The study by Wallentin et al. has shown that clopidogrel resistance can be overcome by ex vivo addition of its active metabolite, which resulted in high platelet inhibition regardless of the initial platelet reactivity both after 600 mg loading dose and 75 mg maintenance dose of clopidogrel [23]. This suggests that variable antiplatelet response to clopidogrel depends mainly on plasma levels of the active metabolite and less likely on the platelet receptor properties.

Several other gene polymorphisms may influence clopidogrel action. However, the results of studies performed in various populations are inconclusive [14-22, 26]. We excluded patients with dual P2Y12 and CYP2C19 polymorphism from the study group, because those coexisting polymorphisms had been previously shown to influence platelet function on clopidogrel in the same population [26]. Of the remaining patients many showed inadequate platelet response to antiplatelet therapy including clopidogrel despite a non-significant difference in median platelet response in CADP test on clopidogrel treatment in subgroups with single P2Y12 or CYP2C19 polymorphisms and controls. Therefore, we found it possible that a third polymorphism such as C3435T of the MDR-1 gene may play a significant role in the variations within the subgroup in platelet response to clopidogrel and aspirin.

Table IV. Outcome in the studied population according to MDR-1 genotype

MDR-1 genotype	Patients without outcome event (n = 88)	Patients with outcome event (n = 10)
TT, n (%)	18 (20.5)	3 (30.0)
CT, n (%)	44 (50)	6 (60.0)
CC, n (%)	26 (29.5)	1 (10.0)
$\chi^2 = 1.80$, p = 0.40		

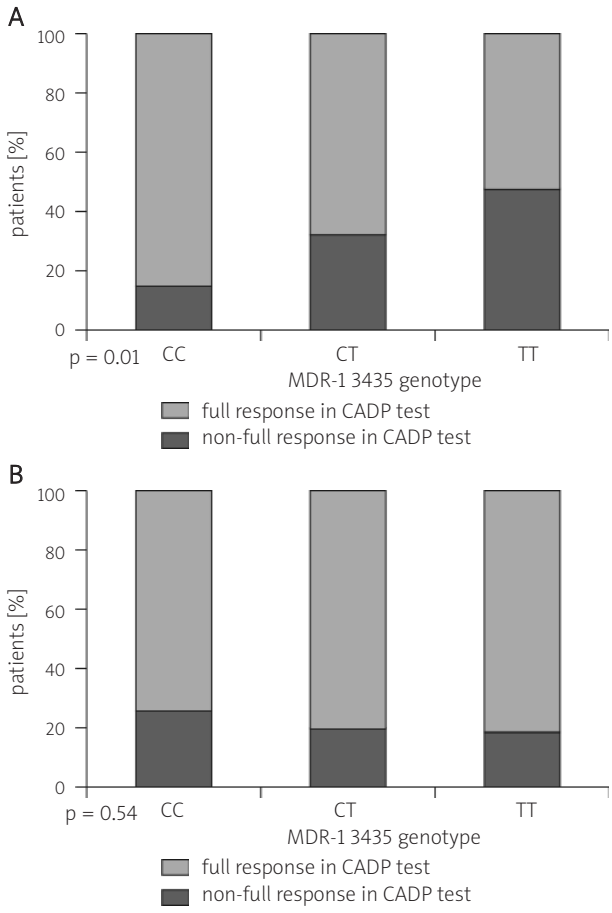

Figure 1. Frequency of non-full response in CADP test (**A**) and CEPI (**B**) according to genotype

Recently, Heestermans et al. have demonstrated that ST elevation myocardial infarction (STEMI) per se leads to impaired oral bioavailability of clopidogrel [44]. In our work the vast majority of patients it the whole group (81.6%) had STEMI. Despite that our results remained unchanged when we limited the analysis to patients with STEMI (data not shown).

Our study did not show any significant difference in outcome in relation to C3435T genotype. This might be due to the relatively small sample size and low frequency of cardiovascular events in the studied population. We performed our study in ACS hospital survivors, which limits the rate of analysed events to the post-discharge period. We excluded patients with concomitant glycoprotein IIb/IIIa inhibitor administration, which also limits the study group to a lower risk population. However, our results are consistent with the published data from a recent large study [13].

Limitations of study

We used only one method, with a point-of-care device, for measurement of platelet function. The use of PFA-100 to measure platelet response to clopidogrel treatment remains questionable [28]. However, it seems unlikely that the results observed in this and previous studies were based only on accidental changes in closure times measured by PFA-100. Nevertheless, our results should be verified with other available methods used for determination of response to antiplatelet agents. Second, only one polymorphism of the MDR-1 gene has been studied, whereas other polymorphisms of this gene might be of relevance [24, 39]. We did not perform an analysis of other known polymorphism (G2677T, C1236T) because the small size of the study group would not allow powerful statistical calculations. However, their influence, especially when linked in haplotypes, cannot be excluded. We are aware of limitations of the genetic analysis which we performed, using restriction endonuclease instead of the gold standard represented by DNA sequencing. Our results should be verified using this technique.

We were unable to analyse plasma levels of clopidogrel and its active metabolite. Further studies evaluating the influence of MDR-1 gene polymorphism on antiplatelet effect of clopidogrel should include these measurements in its protocol. In addition, we did not measure von Willebrand factor concentration, which is known to influence PFA-100 closure times [28].

Finally, some other drugs can influence P-glycoprotein activity including those (for example digoxin and nifedipine derivatives) used in treatment of patients with cardiovascular system diseases [25]. However, digoxin, which is the substrate of P-glycoprotein and therefore could compete with clopidogrel, is not used in the acute phase of ACS, the time when the platelet function was assessed. Although there are reports of a significant interaction between calcium channel blockers and antiplatelet effect of clopidogrel, calcium channel blockers are not recommended as part of treatment of the acute phase in patients with ACS, and therefore were not used in the present study. However, we cannot exclude interaction of other substrates of P-glycoprotein with clopidogrel used by patients before the onset of ACS.

Conclusions

Our study showed that C3435T polymorphism of the MDR-1 gene influences platelet reactivity as measured by PFA-100 but it does not interact with prognosis in patients with ACS. This result should be confirmed in other populations, with other available methods, because high frequency of the TT genotype associated with impaired inhibition of ADP-dependent platelet activation may diminish treatment benefits in a substantial group of patients receiving antiplatelet therapy.

References

1. Van de Werf F, Bax J, Betriu A, et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation. *Eur Heart J* 2008; 29: 2909-45.
2. Bassand JP, Hamm CW, Ardissino D, et al. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. Guidelines for the diagnosis and treatment of non-ST-segment elevation acute coronary syndromes. *Eur Heart J* 2007; 28: 1598-660.
3. Gurbel PA, Tantry US. Clopidogrel resistance? *Thromb Res* 2007; 120: 311-21.
4. Reny JL, De Moerloose P, Dauzat M, et al. Use of the PFA-100 closure time to predict cardiovascular events in aspirin-treated cardiovascular patients: a systematic review and meta-analysis. *J Thromb Haemost* 2008; 6: 444-50.
5. Crescente M, Di Castelnuovo A, Iacoviello L, et al. Response variability to aspirin as assessed by the platelet function analyzer (PFA)-100. A systematic review. *Thromb Haemost* 2008; 99: 14-26.
6. Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. *Eur Heart J* 2006; 27: 2420-5.
7. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006; 48: 1742-50.
8. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J Thromb Haemost* 2006; 4: 542-9.
9. Bliden KP, DiChiara J, Tantry US, et al. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: is the current antiplatelet therapy adequate? *J Am Coll Cardiol* 2007; 49: 657-66.
10. Buonamici P, Marcucci R, Migliorini A, et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. *J Am Coll Cardiol* 2007; 49: 2312-7.
11. Huczek Z, Filipiak KJ, Kochman J, et al. Baseline platelet reactivity in acute myocardial infarction treated with primary angioplasty

– influence on myocardial reperfusion, left ventricular performance, and clinical events. *Am Heart J* 2007; 154: 62-70.
12. Malek LA, Spiewak M, Filipiak KJ, et al. Persistent platelet activation is related to very early cardiovascular events in patients with acute coronary syndromes. *Kardiol Pol* 2007; 65: 40-5.
13. Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009; 360: 363-75.
14. Frere C, Cuisset T, Morange PE, et al. Effect of cytochrome p450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. *Am J Cardiol* 2008; 101: 1088-93.
15. Giusti B, Gori AM, Marcucci R, et al. Role of glycoprotein Ia gene polymorphisms in determining platelet function in myocardial infarction patients undergoing percutaneous coronary intervention on dual antiplatelet treatment. *Atherosclerosis* 2008; 196: 341-8.
16. Fontana P, Senouf D, Mach F. Biological effect of increased maintenance dose of clopidogrel in cardiovascular outpatients and influence of the cytochrome P450 2C19*2 allele on clopidogrel responsiveness. *Thromb Res* 2008; 121: 463-8.
17. Giusti B, Gori AM, Marcucci R, et al. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. *Pharmacogenet Genomics* 2007; 17: 1057-64.
18. Fontana P, Hulot JS, De Moerloose P, et al. Influence of CYP2C19 and CYP3A4 gene polymorphisms on clopidogrel responsiveness in healthy subjects. *J Thromb Haemost* 2007; 5: 2153-5.
19. Cuisset T, Frere C, Quilici J, et al. Role of the T744C polymorphism of the P2Y12 gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. *Thromb Res* 2007; 120: 893-9.
20. Lev EI, Patel RT, Guthikonda S, et al. Genetic polymorphisms of the platelet receptors P2Y (12), P2Y (1) and GP IIIa and response to aspirin and clopidogrel. *Thromb Res* 2007; 119: 355-60.
21. Bernardo E, Angiolillo DJ, Ramírez C, et al. Lack of association between gene sequence variations of platelet membrane receptors and aspirin responsiveness detected by the PFA-100 system in patients with coronary artery disease. *Platelets* 2006; 17: 586-90.
22. Bura A, Bachelot-Loza C, Ali FD, et al. Role of the P2Y12 gene polymorphism in platelet responsiveness to clopidogrel in healthy subjects. *J Thromb Haemost* 2006; 4: 2096-7.
23. Wallentin L, Varenhorst C, James S, et al. Prasugrel achieves greater and faster P2Y12receptor-mediated platelet inhibition than clopidogrel due to more efficient generation of its active metabolite in aspirin-treated patients with coronary artery disease. *Eur Heart J* 2008; 29: 21-30.
24. Taubert D, von Beckerath N, Grimberg G, et al. Impact of P-glycoprotein on clopidogrel absorption. *Clin Pharmacol Ther* 2006; 80: 486-501.
25. Evans WE, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N Engl J Med* 2003; 348: 538-49.
26. Malek LA, Kisiel B, Spiewak M, et al. Coexisting polymorphisms of P2Y12 and CYP2C19 genes as a risk factor for persistent platelet activation with clopidogrel. *Circ J* 2008; 72: 1165-9.
27. Silber S, Albertsson P, Avilés FF, et al. Guidelines for percutaneous coronary interventions. *Eur Heart J* 2005; 26: 804-47.
28. Hayward CP, Harrison P, Cattaneo M, et al. Platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. *J Thromb Haemost* 2006; 4: 312-9.
29. Foussas SG, Zairis MN, Patsourakos NG, et al. The impact of oral antiplatelet responsiveness on the long-term prognosis after coronary stenting. *Am Heart J* 2007; 154: 676-81.
30. Gum PA, Kottke-Marchant K, Poggio ED, et al. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. *Am J Cardiol* 2001; 88: 230-5.
31. Madan M, Berkowitz SD, Christie DJ, et al. Rapid assessment of glycoprotein IIb/IIIa blockade using the platelet function analyzer (PFA-100) during percutaneous coronary interventions. *Am Heart J* 2001; 141: 226-33.
32. Ye S, Dhillon S, Ke X, et al. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res* 2001; 29: E88-8.
33. Jamroziak K, Balcerczak E, Młynarski W, et al. Distribution of allelic variants of functional C3435T polymorphism of drug transporter MDR1 gene in a sample of Polish population. *Pol J Pharmacol* 2002; 54: 495-500.
34. Jamroziak K, Młynarski W, Balcerczak E, et al. Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *Eur J Haematol* 2004; 72: 314-21.
35. Tan EK, Drozdzik M, Bialecka M, et al. Analysis of MDR1 haplotypes in Parkinson's disease in a white population. *Neurosci Lett* 2004; 372: 240-4.
36. Kurzawski M, Droździk M, Suchy J, et al. Polymorphism in the P-glycoprotein drug transporter MDR1 gene in colon cancer patients. *Eur J Clin Pharmacol* 2005; 61: 389-94.
37. Kurzawski M, Pawlik A, Górnik W, et al. Frequency of common MDR1 gene variants in a Polish population. *Pharmacol Rep* 2006; 58: 35-40.
38. Jamroziak K, Balcerczak E, Calka K, et al. Polymorphisms and haplotypes in the multidrug resistance 1 gene (MDR1/ABCB1) and risk of multiple myeloma. *Leuk Res* 2009; 33: 332-5.
39. Aarnoudse AJ, Dieleman JP, Visser LE, et al. Common ATP-binding cassette B1 variants are associated with increased digoxin serum concentration. *Pharmacogenet Genomics* 2008; 18: 299-305.
40. Drescher S, Schaeffeler E, Hitzl M, et al. MDR1 gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine. *Br J Clin Pharmacol* 2002; 53: 526-34.
41. Fellay J, Marzolini C, Meaden ER, et al. Swiss HIV Cohort Study. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002; 359: 30-6.
42. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; 70: 189-99.
43. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; 97: 3473-8.
44. Heestermans AA, van Werkum JW, Taubert D, et al. Impaired bioavailability of clopidogrel in patients with a ST-segment elevation myocardial infarction. *Thromb Res* 2008; 122: 776-81.

Wpływ polimorfizmu C3435T genu *multidrug resistance-1* (MDR-1) na reaktywność płytek krwi i rokowanie u chorych z ostrymi zespołami wieńcowymi

Mateusz Śpiewak[1, 2], Łukasz A. Małek[1], Grażyna Kostrzewa[3], Bartłomiej Kisiel[4], Agnieszka Serafin[2], Krzysztof J. Filipiak[2], Rafał Płoski[3], Grzegorz Opolski[2]

[1] I Klinika Choroby Wieńcowej, Instytut Kardiologii, Warszawa

[2] I Klinika Kardiologii, Warszawski Uniwersytet Medyczny

[3] Klinika Genetyki Medycznej, Warszawski Uniwersytet Medyczny

[4] Klinika Chorób Wewnętrznych i Reumatologii, Wojskowy Instytut Medyczny, Warszawa

Streszczenie

Wstęp: Polimorfizm C3435T genu *multidrug resistance-1* (MDR-1) wpływa na ograniczenie biodostępności klopidogrelu po podaniu doustnym oraz na rokowanie chorych z zawałem serca.

Cel: Ocena wpływu polimorfizmu C3435T genu MDR-1 na aktywność płytek krwi oraz na rokowanie u pacjentów z ostrym zespołem wieńcowym (OZW) leczonych angioplastyką wieńcową z implantacją stentu.

Metody: Grupę 98 pacjentów podzielono na podgrupy w zależności od wyników oznaczeń aktywności płytek w testach z kolagenem i adrenaliną (epinefryną) (CEPI-CT) oraz z kolagenem i dwufosforanem adenozyny (CADP-CT) wykonywanych przy użyciu urządzenia Platelet Function Analyser-100 (PFA-100). Osoby z wynikiem oznaczenia CADP-CT < 130 s i osoby z wynikiem oznaczenia CEPI-CT ≤ 193 s były określane jako pacjenci z niepełną odpowiedzią na leczenie przeciwpłytkowe. Z badania wyłączono chorych ze współistniejącym polimorfizmem genów *P2Y12* i *CYP2C19*. Analizowano częstość występowania następujących zdarzeń: zgonu, zawału serca niezakończonego zgonem oraz udaru mózgu niezakończonego zgonem.

Wyniki: Pacjenci będący homozygotami TT mieli większe prawdopodobieństwo uzyskania wyniku oznaczającego niepełną odpowiedź na leczenie przeciwpłytkowe w teście z ADP w porównaniu z homozygotami CC (OR 5,23; 95% CI 1,34–20,45; p = 0,017). W przypadku heterozygot w porównaniu z homozygotami CC obserwowano słaby trend do częstszego występowania niepełnej odpowiedzi w teście z CADP u chorych z genotypem CT (OR 2,71; 95% CI 0,80–9,14; p = 0,11). Nie zaobserwowano zależności pomiędzy polimorfizmem C3435T genu MDR-1 a odpowiedzią w teście CEPI – ani w przypadku homozygot TT (p = 0,57), ani w przypadku heterozygot (p = 0,55). W okresie obserwacji wynoszącym średnio 1,7 roku nie wykazano wpływu polimorfizmu na częstość występowania złożonego punktu końcowego.

Wnioski: Polimorfizm C3435T genu MDR-1 wpływa na reaktywność płytek zależną od ADP u chorych z OZW, ale nie wpływa na rokowanie średnioterminowe w tej populacji.

Słowa kluczowe: leki przeciwpłytkowe, wielolekooporność, ostre zespoły wieńcowe, polimorfizm, zmienna odpowiedź

Kardiol Pol 2009; 67: 827-834

Adres do korespondencji:

lek. Mateusz Śpiewak, I Klinika Choroby Wieńcowej, Instytut Kardiologii, ul. Alpejska 42, 04-628 Warszawa, tel.: +48 22 343 42 72, e-mail: mspiewak@ikard.pl

Praca wpłynęła: 24.01.2009. **Zaakceptowana do druku:** 07.05.2009.