MANIFESTATIONS OF CYP2C19 POLYMORPHISM IN ACUTE CORONARY SYNDROME PATIENTS THAT UNDERWENT PERCUTANEOUS CORONARY INTERVENTION

Nazgul K. Kulmyrzaeva¹, Gaziza A. Smagulova¹, Galina V. Veklenko¹, Nazgul A. Seitmaganbetova¹, Samat M. Mukanov², Nurzhan S. Biyassilov¹, Ainagul A. Kulniyazova¹, Ainura O. Ashimova¹, Aigul A. Zhaubatyrova¹, Ondasyn M. Aliev¹

Assistant at Propaedeutics of Internal Medicine and Clinical Pharmacology, West Kazakhstan Marat Ospanov State Medical University, Aktobe, Kazakhstan;
Assistant at interventional cardiology department, Emergency Hospital, Aktobe, Kazakhstan

Email: guldana-gulsezim@yandex.ru

Received on 22-05-2016
Accepted on 25-06-2016

Abstract

Background: The purpose of this study is to investigate the rate of CYP2C19 polymorphism manifestation in acute coronary syndrome (ACS) patients that underwent percutaneous coronary intervention (PCI).

Methods: We studied the manifestation of CYP2C19 genotype polymorphism in 100 ACS patients that underwent PCI. The most frequently encountered risk factors included arterial hypertension (in 71% of patients), smoking (in 31% of patients), and obesity (in 27% of patients). It was found that the CYP2C19 *1/2 gene (40%) and CYP2C19 *1/3 gene (10%) carriage was frequently encountered in patients with a two-vessel coronary bed disease. The compliance of clopidogrel was evaluated according to the results of endpoints (thrombosis, death, hemorrhage). The primary endpoint during the monitored period was the occurrence of stent thrombosis.

Results: Stent thrombosis was found in two patients with a two-vessel disease, who were CYP2C19 *2 gene carriers. Early stent thrombosis with a fatal outcome occurred in a patient who was a CYP2C19 *3 gene carrier with a single-vessel coronary bed disease. After six months of observation, fatal outcome was pronounced in five cases, two of which were CYP2C19 *1/*3 carriers, two others were CYP2C19 *1/*2 carriers, while one was a CYP2C19 *1/*1 carrier.

Conclusion: Thus, patients that carried at least CYP2C19 *1/*2 and CYP2C19 *1/*3 were more prone to high platelet reactivity during clopidogrel intake, which was associated with a poor clinical outcome after the coronary stent placement.
Keywords: Genetic Polymorphism; CYP2C19; clopidogrel; acute coronary syndrome; percutaneous coronary intervention.

Introduction
The prevalent pathology among cardiovascular diseases is atherosclerotic lesion of vessels and associated atherothrombosis, which causes more than 28% of deaths worldwide. According to the World Health Organization, 17.3 million people died from cardiovascular diseases in 2008, which amounted to 30% of all lethal cases worldwide. According to the predictions of the World Health Organization, 23.6 million people will have died from cardiovascular diseases by 2030, primarily from heart diseases or stroke, which will remain the only primary causes of death.

According to the information provided by the Committee on Statistics of the Ministry of National Economy of the Republic of Kazakhstan, cardiovascular diseases caused almost 1/3 of all deaths in Kazakhstan in recent years. At present, the main treatment methods in case of coronary artery disease are invasive methods of myocardial revascularization.

Endovascular intervention improve the patients’ quality of life, restore the patients’ working capacity, and increase exercise tolerance.

An important aspect in modern cardiologic practice is the prevention of thrombosis and rethrombosis, which is especially relevant for patients that underwent PCI. At present, clopidogrel is one of the main agents used in antiplatelet therapy. It reduces the rate of thrombotic complications in cardiology patients. During the last 1.5 years, several studies were published that covered the association of loss-of-function CYP2C19*2 (also denoted as 681G>A) polymorphism, with various clinical endpoints of clopidogrel administration. Trenk D. and coauthors (2008) studied whether the loss-of-function CYP2C19*2 polymorphism was associated with high (>14%) residual platelet aggregation on clopidogrel and whether high on-clopidogrel residual platelet aggregation impacts clinical outcome after elective coronary stent placement.

The Circulation journal notes, that according to the results of a study by Mega J.L. and coauthors, knowing the genotype status in addition to other discovered risk factors could be clinically useful for better predicting hemorrhagic complications in patients that are treated with clopidogrel due to coronary stent placement. Numerous studies cover the clinical and laboratory metabolism of clopidogrel in ACS patients and the necessity of gene typing with the determination of polymorphic gene variants, which determine the metabolism of the drug and affect the clinical outcome of patients.
Methods

The present pharmacological study is open, prospective, and cohort. The research protocol was approved by the local ethics committee of the West Kazakhstan M. Ospanov State Medical University by Minutes No. 4 dated October 8, 2013.

Participants of the Study

The study included 100 patients with documented ACS with an implanted drug-eluting stent and prescribed double antiaggregation therapy (aspirin+ clopidogrel). ACS was diagnosed if at least two of the following criteria were met: 1. Typical retrosternal pain. 2. ECG signs of myocardial ischemia or necrosis. 3. Typical increase in the level of serum markers of myocardial necrosis two times above the upper normal boundary. Coronary angiography was done within two days after the onset of symptoms. Coronary stenosis was diagnosed when the vascular diameter reduced by ≥ 50%. Depending on the number of affected coronary arteries, patients were divided into ones with single-vessel, two-vessel, and three-vessel diseases. All patients gave their written informed consent. The consent form was approved by the local university ethics committee.

Collection of Data and Medical Records

All patients that were included in the study were native Kazakh and Slavic dwellers of the Aktobe Region. Demographic data and information about risk factors for all patients were obtained from medical records when the patients were admitted to the interventional cardiology department of the emergency hospital and the medical center of the West Kazakhstan State Medical University in Aktobe, Kazakhstan, from October 2014 till March 2015. Arterial hypertension patients included persons with high blood pressure (> 140/90 mm Hg), measured three times, or if patients were already administered antihypertensive drugs. Patients with diabetes mellitus included persons with a fasting blood glucose level of 126 mg/dL (6.1 mmol/L) or those who have already received antidiabetic treatment. A family history was diagnosed in cases of premature myocardial infarction or sudden cardiac death of close relatives (for instance, parents, siblings or children). The smoking status was determined as follows: regular smoker if the participant regularly smoked one cigarette per day and/or one cigar or one pipe per week. Participants who used to smoke one cigarette per day and/or one cigar or one pipe per week were considered former smokers. Never smokers were persons who never smoked tobacco products regularly. Participants were considered recently quit smokers if they had not smoked for six months before their inclusion in the study. Depending on the ACS type, patients were divided into groups with myocardial infarction with ST-segment elevation and without ST-segment elevation; unstable angina.
was diagnosed if the level of biochemical markers of necrosis was within normal values. Patients who were initially hospitalized with unstable angina, but who later developed myocardial infarction when in hospital, were included into the myocardial infarction group.

Inclusion criteria were as follows: ACS with ST-segment elevation and ACS without ST-segment elevation, patient’s informed consent, age from 18 to 90. Exclusion criteria were as follows: cardiomyopathy, heart disease, severe somatic or neurological disorders, patient’s dissent, violation of the dosing regimen (aspirin and clopidogrel).

Upon inclusion, the drug treatment was corrected in accordance with international recommendations, if needed. All patients were prescribed a daily dose of 75 mg clopidogrel and 100 mg aspirin. In case of no contraindications, all patients were recommended taking Beta-blockers, angiotensin-converting-enzyme inhibitors, and statins, in addition to the antiaggregation therapy.

**Blood sampling and gene amplification**

The venous blood of ACS patients was sampled after they took a loading dose of clopidogrel (300 or 600 mg). Blood samples for DNA extraction were collected in 3 ml tubes containing potassium EDTA. Total genomic DNA was extracted from peripheral blood leukocytes by the salting-out method [22]. Two single nucleotide CYP2C19*2 (G681A, rs4244285) and CYP2C19*3 (G636A, rs4986893) polymorphisms were evaluated in each patient by commercial Taqman probes (Applied Biosystems, Paisley, UK) on an ABI 7900HT Fast RealTime PCR Thermocycler (Foster City, California, USA) in the laboratory of Molecular Cardiology of the Institute of Cardiology Lithuanian University of Health Sciences. Real-time PCR was carried out in a total volume of 25 µl containing 12.5 µl of Universal PCR Master Mix (Applied Biosystems, Foster City, California, USA), 10.25 µl of PCR Grade water (Ambion, Carlsbad, California, USA). 1.25 µl of each Taqman probe and 10-30 ng of genomic DNA was used per reaction. Amplification was done according to the manufacturer’s protocol for validated probes: 10 min at 95°C, followed by 40 cycles: 15 s at 95°C and 30 s at 60°C.

**Statistical analysis**

Data on age and cholesterol, glucose, and creatinine levels were presented as a mean value ±SD. The ratio of genotypes were presented in percentages. The differences of measurable variables between the groups were analyzed by unpaired Student’s t-test. The differences of categorical variables, including smoking, hypertension, DM, impaired left ventricular (LV) function (if LV ejection fraction < 45%), and mutations of each genotype, were analyzed by Fisher’s exact test. Chi-squared analyses were used to test deviations of genotype distributions from the Hardy-
Weinberg equilibrium and to determine allele or genotype ratios. The risk factors that appeared to be possible significant predictors (p<0.2) in the multiple logistic regression analysis. Cox proportional hazard models were used to evaluate the effects of genetic polymorphism and other clinical variables in event-free survival. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. All statistical analyses were performed using SPSS Advanced Statistics 10.0 for Windows. In this study, a value of P<0.05 was taken as statistically significant.

**Literature Survey**

Since 2010, the manufacturer of the original clopidogrel (Plavix) and the USA Food and Drug Administration have considered it necessary to draw the attention of practitioners to the possibility of detecting patients with weak reaction to clopidogrel by gene typing or evaluating the residual platelet reactivity. Also of note is the availability of clinical genetic testing systems that determine the allele variants of the CYP2C19 enzyme as the most important one in the clopidogrel metabolism.

Several recent studies investigated whether the loss-of-function CYP2C19*2 polymorphism was associated with the clinical outcomes of clopidogrel use.

The low activity of the enzyme is associated with a risk of myocardial infarction or ischemic stroke. According to the TRITON-TIMI 38 trial, patients with reduced enzyme activity were at 53% higher risk of death from cardiovascular events. In addition, some gene variants could be associated with risk of thrombosis of placed stents.

**Results**

The study included 100 ACS patients (average age was 61), most of whom were male. The clinical and demographic characteristics and the rate of the main risk factors is presented in Table 1.

**Table 1.Clinical and demographic characteristics of patients.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>UA</th>
<th>MI without ST</th>
<th>MI with ST</th>
<th>Total ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>50</td>
<td>32</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Age</td>
<td>57.7(9.9)</td>
<td>64.2(11.2)</td>
<td>61.5(14)</td>
<td>61.28(11.4)</td>
</tr>
<tr>
<td>Male/female</td>
<td>29/21</td>
<td>22/10</td>
<td>13/5</td>
<td>64/36</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>42(84%)</td>
<td>22(69%)</td>
<td>7(39%)</td>
<td>71(%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2(4%)</td>
<td>11(34%)</td>
<td>2(11.1%)</td>
<td>15(15%)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.83(1.5)</td>
<td>4.42(1.3)</td>
<td>8.7(7.2)</td>
<td>5.33(3.3)</td>
</tr>
<tr>
<td>Smoking</td>
<td>15(30%)</td>
<td>6(19%)</td>
<td>11(61.1%)</td>
<td>32(32%)</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>--------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Obesity ≥25 kg/m²</td>
<td>15(30%)</td>
<td>4(12.5%)</td>
<td>6(33%)</td>
<td>25%</td>
</tr>
<tr>
<td>Obesity ≥30 kg/m²</td>
<td>12(24%)</td>
<td>9(28.1%)</td>
<td>6(33%)</td>
<td>27%</td>
</tr>
<tr>
<td>Troponin T (qualitative)</td>
<td>5(10%)</td>
<td>9(28.1%)</td>
<td>18(100%)</td>
<td>32</td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>27(54%)</td>
<td>8(25%)</td>
<td>9(50%)</td>
<td>44(44%)</td>
</tr>
<tr>
<td>Two-vessel disease</td>
<td>7(14%)</td>
<td>5(15%)</td>
<td>1(6%)</td>
<td>13(13%)</td>
</tr>
<tr>
<td>Three-vessel disease</td>
<td>19(38%)</td>
<td>16(50%)</td>
<td>8(44%)</td>
<td>43(43%)</td>
</tr>
</tbody>
</table>

Conventional risk factors (arterial hypertension, diabetes mellitus, hypercholesterolemia, smoking, and obesity) were assessed for all patients. The prevailing risk factors were arterial hypertension (in 71% of patients), smoking (in 31% of patients), and obesity (in 27% of patients).

A total of 100 patients underwent PCI. The most frequent diagnoses were unstable angina (50%) and myocardial infarction without ST-segment elevation (MI without ST) (32%). Troponin was positive in 100% of cases of myocardial infarction with ST-segment elevation (MI with ST). On average, PCI was done 1-2 days after the onset of the index event. Coronarography found single-vessel disease in 44 patients, two-vessel diseases in 13 patients, and three-vessel disease in 43 patients. All patients underwent angioplasty with a stenting of the infarction-associated artery regardless of the number of affected vessels. The rate of anterior interventricular branch disease was 89%; the rate of circumflex branch disease was 62%; the rate of right coronary artery disease was 56%. Angiographic success was achieved in all patients and was accompanied by a clinical effect – disappearance or reduced rate of angina attacks or rapid positive dynamic of acute myocardial infarction. All patients took clopidogrel at a loading dose of 300 mg, 75 mg for 12 months.

Clopidogrel is one of the most frequently prescribed drugs for ACS patients and/or after PCI. However, the reactions to clopidogrel vary, while the ADP-induced thrombocyte aggregation could vary significantly \(^7\). For instance, an attempt was made to investigate whether the loss-of-function CYP2C19 681G>A *2 polymorphism is associated with high (>14%) residual platelet aggregation (RPA) on clopidogrel and whether high on-clopidogrel RPA impacts clinical outcome after elective coronary stent placement \(^2\). It was found that patients carrying at least one CYP2C19*2 allele were more prone to high-on clopidogrel platelet reactivity, which is associated with poor clinical outcome after coronary stent placement. Often encountered were variants of this gene that code the formation of an enzyme with a reduced or lost function. Loss-of-function polymorphism is indicated as CYP2C19*2, while ordinary polymorphism

\[^7\]
The polymorphic variants of the CYP2C19 gene in patients after stenting are presented in Table 2. Several studies found that CYP2C19 *2 heterozygotes and homozygotes had fewer active clopidogrel metabolites and, consequently, higher platelet aggregation, compared to homozygote *1 aggregation \(^6-10\). In addition, there is serious evidence that links the CYP2C19 genotype with the clinical outcomes of ACS patients, especially of those that underwent PCI or clopidogrel treatment \(^3,9-13\), probably due to reduced formation of active clopidogrel metabolites. The most comprehensive studies that associated the CYP2C19 genotype with the reaction to clopidogrel mostly included ACS patients, almost all of whom underwent PCI.

Extensive meta-analyses found that ACS patients that underwent PCI and took clopidogrel and were CYP2C19*2 hetero- or homozygotes were at high risk of main adverse cardiovascular events, compared to *1 homozygotes (hazard ratio (HR) = 1.55, 95% confidence interval (CI) = 1.11-2.17 for heterozygotes; HR = 1.76, 95% CI = 1.24-2.50 for homozygotes) and higher risk of stent thrombosis (HR = 2.67, 95% CI = 1.69-4.22 for heterozygotes; HR = 3.97, 95% CI = 1.75-9.02 for homozygotes) \(^11\).
Discussion

The primary endpoint during the monitored period was three cases of early stent thrombosis. Stent thrombosis was diagnosed in two patients with two-vessel disease, who carried the CYP2C19 *2 gene. Early stent thrombosis with a fatal outcome was diagnosed in a carrier of the CYP2C19 *3 gene with single-vessel coronary bed disease. The diagnosis was confirmed morphologically and by angiography. Mega J.L. and coauthors conducted a meta-analysis of studies that evaluated the association between the loss-of-function CYP2C19 alleles (mostly *2) and the results of observation of clopidogrel-treated patients. Additional meta-analyses confirmed the association between CYP2C19 and stent thrombosis with an odds ratio from 1.75 to 3.82 among *2 heterozygotes and homozygotes. Carriers of the loss-of-function CYP2C19 allele were at 50% higher risk of adverse cardiovascular events and at almost three times higher risk of stent thrombosis during clopidogrel treatment. Meta-analysis data showed that genetic testing enabled detecting a considerable number of patients (up to 30% of the total number), for whom clopidogrel offers insufficient protection from recurrent ischemic events. This is also confirmed by the results of the present study. Fatal outcome was pronounced in five cases, six months after the observation – two of them were CYP2C19 *1/*3 carriers, two others were CYP2C19*1/ *2 carriers, and one was a CYP2C19 *1/*1 carrier. In three cases, the cause of death was recurrent myocardial infarction; in two cases, sudden cardiac death was confirmed morphologically. Secondary endpoints were adverse cardiovascular events and hemorrhages. A total of four cases of hemorrhage, nosebleed, and gastrointestinal bleeding were registered. No large hemorrhages were registered.

Giusti B. and coauthors also studied the relation of CYP2C19*2 polymorphism to the occurrence of stent thrombosis within a 6-month follow-up in patients undergoing percutaneous coronary interventions with drug-eluting stent implantation on dual-antiplatelet treatment enrolled in the RECLOSE trial. Another endpoint of the present study was composite and included stent thrombosis and “cardiac death”. Apart from genetic factors, clopidogrel metabolism is also affected by such conditions as comorbidity, drugs, food characteristics, age, and way of life.

Thus, patients that carried at least CYP2C19 *1/*2, CYP2C19 *1/*3 were more prone to high platelet reactivity during clopidogrel intake, which was associated with a poor clinical outcome after coronary stent placement. The novelty of this study is that it carried out a pharmacogenetic investigation of ASC/PCI clopidogrel-treated patients that lived in a region inhabited by Kazakhs and Slavs. The study and its results show that:

1. Carriage of the CYP2C19 *1/*1 gene is frequently encountered among ASC patients.
2. Carriage of the CYP2C19*1/*2 and CYP2C19*1/*3 genes is frequently encountered among ASC patients with a two-vessel disease.

3. A clinical outcome (death, stent thrombosis) was diagnosed in patients that carried the CYP2C19*1/*2 and CYP2C19*1/*3 genes.

The results of this study prove the necessity of the genetic investigation of ASC patients, with a view to determining the level of clopidogrel metabolism and preventing adverse clinical outcomes.

Acknowledgements

The authors would like to thank the Novosibirsk State University for providing premises and equipment for this research. Authors declare that they have no conflict of interest and sources of funding. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants.

References


Corresponding author:
Nazgul A. Seitmaganbetova*,
Email: guldana-gulsezim@yandex.ru