JMS 2022; Vol 5(3):78-84
UDC:615.273.53.036:[616-056.7:575.22
DOI:10.55302/JMS2253078s

TYPE OF CYP450 2C19 GENE METABOLIZERS IN PATIENTS UNDERGOING PERCUTANEOUS CORONARY INTERVENTION IN THE REPUBLIC OF NORTH MACEDONIA- A PILOT

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Abstract

Clopidogrel has an antiplatelet effect, which is used to reduce the risk of myocardial infarction and stroke in high-risk patients in the context of primary prophylaxis. In the body it is subjected to metabolism by the liver enzyme system CYP450.

In this project, laboratory conditions have been established to determine the polymorphisms of the CYP450 2C19 gene.

The structure of the polymorphisms in the CYP450 2C19 gene and the type of metabolizer has been determined.This study included 42 heart patients with St. post PCI, mean age 65-9.7 years, treated with 75 mg Clopidogrel daily.

The following polymorphisms were examined: c.681G> A; c.636G> A; c.1A> G; c.1297C> T; c.395G> A; c.819 + 2T> A; c.358T> C; c.-806C> T.

Appropriate laboratory conditions have been established for the precise determination of polymorphisms in the CYP450 2C19 gene and the determination of the type of metabolizer.

The results showed that in our patients the largest number of polymorphisms occur at the position c.681G> A which leads to the creation of an intermediate metabolizer, while the polymorphism at the position c.-806C> T leads to the creation of an ultrafast metabolite.

The largest number of polymorphisms occurs in c.681G> A CYP450 2C19 gene, which leads to the creation of an intermediate metabolizer.

Because of this, and before starting the therapy in patients St. post PCI, it is necessary to first determine what clopidogrel metabolizers they are and whether they possess LF or GF alleles.

Key words: clopidogrel, metabolite, polymorphisms, CYP450 2C19 gene.

Introduction

Clopidrogel, known under the trademarked name Plavix, is an antiplatelet drug used to reduce the risk of myocardial infarction and stroke in high-risk patients, in the context of primary prophylaxis.

This drug is also used in the secondary prevention of new atherosclerotic events in patients with atherosclerotic disease, who have had a previous myocardial infarction and/or stroke, as well as in patients with peripheral arterial disease [1-5].

The drug is taken orally, and in the body it undergoes metabolism by the liver enzyme system CYP450, also known as cytochrome P450 [5].

Cytochrome P450 (CYP) is an enzyme system that forms a polymorphic superfamily of enzymes involved in the metabolism of various exogenous and endogenous substances in the body [6-7].

There are 57 known CYP genes in the human genome that encode functional enzymes, grouped into 18 protein families and designated as CYP1-18. According to their function, these enzymes are mainly monooxygenases that are involved in the metabolism of various xenobiotics, including certain drugs. CYP1-3 families are involved in the metabolism of exogenous substances, such as the metabolism of drugs [7].

The cytochrome P450 2C19 gene, which encodes the 2C19 protein, has been identified as important in the metabolism of clopidogrel [5].

It is estimated that enzymes of the 2C family, including enzyme 2C19, represent about 20% of hepatic P450, and that specifically enzyme 2C19 is involved in the metabolism of at least 10% of drugs in clinical use today. The 2C19 gene is located in the P450 gene cluster with chromosomal location 10q24 [5-7].

Clopidrogel is a pro-drug, which after entering the body goes through two stages of activation (metabolic conversion into an active drug). CYP2C19, CYP1A2 and CYP2B6 are involved in the first step, and CYP2C19, CYP2C9, CYP2B6 and CYP3A are involved in the second step of metabolic activation.

Activated metabolites of clopidrogel bind to the ADP receptor, that is, they specifically bind irreversibly to the P2Y12 receptor subtype and inhibit its function in platelet activation [6-8].

To date, over 2000 polymorphisms in the CYP2C19 gene have been described and cataloged in the Human CYP Allele Nomenclature Database [9].

Different variants (alleles) of CYP2C19 encode a CYP2C19 protein that has different enzymatic activity. Some alleles have higher, and others have lower enzyme activity and through the role in the metabolism of clopidogrel in the body they determine their activity (and indirectly efficiency and safety). The nomenclature for the various polymorphic variants (or alleles) consists of the name CYP2C19, followed by the symbol * and a number.

The most common variant is the CYP2C19*1 allele, also known as the wild allele, and the alleles that are significantly associated with interindividual differences in the pharmacokinetics of various drugs are CYP2C19*2, CYP2C19*3, CYP2C19*17 [9-11].

CYP2C19*2 (NM_000769.2:c.681GA; p.Pro227Pro; rs4244285) and CYP2C19*3 (NM_000769.2:c.636G>A; p.Trp212Ter; rs4986893) alleles are more common in Asian populations where their frequency is about 15-20%, than in European populations where the frequency is about 3-5% (1 in 20 to 1 in 30 people) [10-12].

These alleles are characterized by reduced enzyme function, and as such are associated with inadequately low metabolization of substrates (such as clopidogrel), leading to reduced activation and therapy failure in humans who carry these alleles [12-14].

The CYP2C19*17 allele (NM_000769.2:c.-806C>T; rs12248560) is gain-of-function, that is, the gene polymorphism leads to increased metabolic activity. The frequency of this allele is about 20% in European and African populations [10, 12].

Based on diplotypes (the combination of both CYP2C19*17 alleles in one person) people are grouped into 5 groups (13-15):

1. Ultrafast metabolizers with increased inhibition of platelets and reduced residual platelet aggregability (*17/*17),
2. Normal metabolizers with increased platelet inhibition and reduced residual platelet aggregability (*1/*17),
3. Normal metabolizers with normal platelet inhibition and normal residual platelet aggregability (*1/*1),
4. Intermediate metabolizers with reduced platelet inhibition and increased residual platelet aggregability and increased risk for cardiovascular episodes (*1/*2, *1/*3, *2/*17), and
5. Slow metabolizers with significantly reduced platelet inhibition and increased residual platelet aggregability and increased risk for cardiovascular episodes (*1/*2, *1/*3, *2/*17).

The frequency of people who have the alleles associated with low metabolic activation, or so-called low metabolizers, is about 14% (or 1 in 7 people).

The clear clinical repercussion in terms of therapy failure associated with these alleles with reduced metabolic activity led in 2010 the US Food and Drug Administration to mandate the addition of a so-called black rectangle in the instructions for use of clopidrogel warning of this association.

The black rectangle indicates that homozygotes for non-functional CYP2C19*2 alleles (2-14% of the US population) at recommended doses will have less of the active metabolite and less reduction in platelet aggregation and advises testing patients for CYP2C19*2 polymorphisms.

The guideline recommends the use of an alternative antiaggregation drug in patients who are homozygous for nonfunctional alleles [16].

Data supporting this practice derive from a clinical study examining the pharmacokinetics and antiplatelet response of clopidogrel in 40 healthy subjects, 10 in each of the known ultrarapid, extensive, intermediate, and slow metabolizer groups.

The study showed reduced exposure to the active metabolite of clopidogrel and increased platelet aggregability in poor metabolizers compared to other groups. Administration of higher doses of clopidogrel to poor metabolizers in the study resulted in increased exposure to the active metabolite and decreased platelet aggregability [2].

To create laboratory conditions for determining polymorphisms of the CYP450 2C19 gene. To determine the structure of polymorphisms in CYP450 2C19 gene in cardiac patients with St. post PCI, as well as to determine the type of metabolizer.

Material and methods

All test subjects included in this study come from different parts of the Republic of North Macedonia.

The examined group is from samples of patients who are treated at PHI University Clinic for Cardiology - Skopje, with a diagnosis of St. post PCI. The criteria for inclusion and exclusion of patients in/from a particular disease are defined by the clinician, who sends the patients for analysis.

All laboratory tests were performed at the Institute of Immunobiology and Human Genetics at the Faculty of Medicine in Skopje.

Blood samples for DNA analysis were taken after patients signed a written consent for donation and storage of DNA sample.

DNA was isolated from peripheral blood leukocytes (7 mL with EDTA) with ready-made isolation kits from MagCore automated nucleic acid extractor - Super with magnetic particles from RBCBioscience and stored in the Macedonian Bank for Human DNA [17].

It features pre-programmed protocols and unique magnetic bit technology, ensuring efficient and consistent purification of nucleic acids. At the same time, it is an instrument that is a combination of an extractor and a spectrophotometer, which enables immediate measurement of the purity and concentration (yield) of the final eluate from the DNA sample.

The gene sequence is then amplified by polymerase chain reaction in a biotin-labeled single (multiplex) reaction. The amplified product is selectively hybridized to strips containing allele-specific (wild-type and mutated) oligonucleotide probes immobilized in an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and a colored substrate.

If no positive result is obtained for any allele of the CYP450 2C19 gene, such a sample is designated as a "blank sample" and as such is included for further analyses. Resolving such cases (empty instances) is beyond the scope of this paper.

Results and discussion

a) Descriptive analysis

The examined group is from samples of patients who are treated at PHI University Clinic for Cardiology - Skopje, with a diagnosis of St. post PCI.

The criteria for inclusion and exclusion of patients in/from a particular disease are defined by the clinician, who sends the patients for analysis. A total of 42 patients were examined, of which 11 were female and 32 were male (Figure 1).

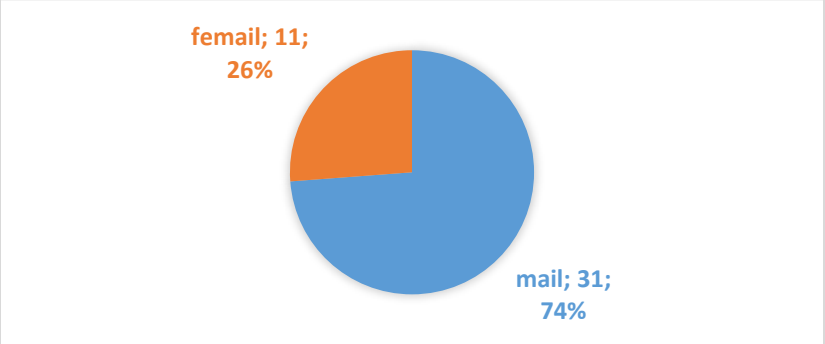

Figure 1. Structure of patients by gender.

The average age of the patients was 65 years. The duration of therapy with 75 milligrams of Clopidogrel averaged about 8.5 months (from 1 to 52 months). Of these, 8 patients, in addition to the basic therapy with 75 mg of Clopidogrel per day, also received 100 mg of Aspirin once a day. None of the patients had an embolic attack while taking the therapy (Table 1).

Several studies have suggested that the CYP2C19 gene is the only one independently associated with variable inhibitory platelet response to clopidogrel [18, 19].

This gene, which is involved in both rates of conversion of clopidogrel to its active metabolite, is highly polymorphic, with some of the polymorphisms can lead to loss of function (LF), and some to gain of function (GF) [12].

The most common alleles associated with LF are *2 and *3. As many as 30% of the white population, 40% of the black population, and 55% of the Asian population are thought to have these variants [20].

However, it is considered that the *2 variant, by itself, is responsible for only 12% of the variation in the platelet response [18, 19].

With the help of additional factors, its participation increases even above 70% [18].

The remaining variants *4, *5, *6, *7, and *8 associated with LF are relatively rarer with a prevalence below 1% [13].

Individuals who are heterozygous for LF-associated polymorphisms are considered intermediate metabolizers of clopidogrel, while homozygotes for these polymorphisms are considered poor metabolizers. The *17 variant, located in the promoter region, is associated with GF [13-15].

However, due to the fact that the *2 variant is missing in the individuals in whom this variant is found, it is not known whether the entire GF is due to *17 only or a part may also be due to the lack of the *2 variant [21].

The examination of polymorphisms in the alleles of the CYP2C19 gene by the RLS method, in the set of our laboratory conditions, showed the following frequency (Table 1):

Table 1. Polymorphisms of the CYP2C19 gene

Polymorphism	Homozygous wild type	Heterozygous	Homozygous mutated
c.681G>A	30 (71.43%)	12 (28.57%)	0 (0%)
c.636G>A	42 (100%)	0 (0%)	0 (0%)
c.1A>G	42 (100%)	0 (0%)	0 (0%)
c.1297C>T	42 (100%)	0 (0%)	0 (0%)
c.395G>A	42 (100%)	0 (0%)	0 (0%)
c.819+2T>A	42 (100%)	0 (0%)	0 (0%)
c.358T>C	42 (100%)	0 (0%)	0 (0%)
c.-806C>T	41 (97,62)	0 (0%)	1 (2,38%)

The percentage representation of phenotypes according to CYP2C19 genotype is: 5-10% ultra-rapid metabolizer (UM), 35-50% extensive metabolizer (EM), 18-45% intermediate metabolizer (IM) and 2-15% poor metabolizer (PM).

According to the Guidelines provided by the Clinical Pharmacogenetics Implementation Consortium, the recommended dose of clopidogrel for patients with St. post PCI and with UM or EM phenotype noncarriers of the LF allele is 75 mg per day.

For patients with PM or IM phenotype, carriers of LF alleles, it is recommended to increase the dose of clopidogrel to 225 mg daily, but also to include alternative therapy with prasugrel and ticagrelor [13].

Such knowledge, over time, also enabled the development of methods for planning the type of therapy, based on the type of metabolizer, the presence or absence of alleles for LF or GF, as well as the increased reactivity of thrombocytes [22, 23].

This representation of the combinations of polymorphisms in the alleles of the CYP2C19 in gene in our examined group showed that the majority of the examined patients belonged to the group of extensive and intermediate metabolizers (Table 2):

Table 2. Type of metabolizer

Type of metabolizer	Number (percentage)
ultra-rapid	1 (2.38%)
extensive	29 (69.05%)
intermediate	12 (28.57%)
poor	0 (0%)

The obtained results of this study represent one of the first that refer to the population in the Republic of North Macedonia.

Our examined sample showed that in our population 69.05% belong to extensive metabolizers, which is a slightly higher percentage than the one described in the literature. Medium metabolizers account for 28.57%, which is in accordance with literature data.

The number of ultra-fast metabolizers is 2.38%, which is below the world average. We did not determine the presence of poor metabolizers in our examined sample. Such obtained results are most likely due to the small size of the examined sample.

The established laboratory conditions allow a new approach to the therapy of patients with St. post PCI in our country.

This enables the development of new protocols for determining the size of the dose of clopidogrel, as well as determining other additional alternative therapy, with the aim of preventing the occurrence of additional complications.

Conclusion

The largest number of polymorphisms occurs in c.681G> A CYP450 2C19 gene, which leads to the creation of an intermediate metabolizer.

Because of this, and before starting the therapy in patients St. post PCI, it is necessary to first determine what clopidogrel metabolizers they are and whether they possess LF or GF alleles.

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