Research Article

Gene polymorphism of CYP2C9 in patients with ischemic heart disease and its significance in the manifestation of genoprotective properties of meldonium

Olesya V. Romaschenko[1]*, Eduard A. Snegin[1], Nina I. Zhernakova[1], Andrey A. Shaposhnikov[1], Lyudmila R. Zakirova[1], Vadim V. Rumbesht[1], Galina A. Batischeva[2]

ABSTRACT

Introduction: The work was carried out in the direction of personalized medicine - an individual approach to the appointment of a metabolic corrector meldonium to patients with ischemic heart disease (IHD) on the basis of determining the genetic characteristics of patients.

Research Tasks: To study of the polymorphism of the cytochrome CYP2C9*2 C430T gene in patients with IHD and to evaluate the significance of this factor in the manifestation of the genoprotective properties of meldonium in this category of patients.

Methods: A study of 90 patients with IHD: Stable angina pectoris was performed. The polymerase chain reaction was used to determine the gene polymorphisms of cytochrome CYP2C9*2 C430T. By the method of DNA comets, according to the method developed by us in *in vitro* tests, the effect of meldonium on the DNA of blood leukocytes of patients was evaluated.

Results: The presence of gene polymorphisms by the alleles CYP2C9*2 C430T was found. They revealed a more severe clinical condition of patients with pathological genes by the alleles CYP2C9*2 C430T in connection with the apparent decrease in the enzymatic activity of cytochrome. Potential genotoxicity of meldonium was revealed when it was administered to patients with normal cytochrome CYP2C9*2 C430T (CC) genotype, and to the genoprotective effect of meldonium when administered to patients with the pathological CYP2C9*2 C430T genotype (CT genotype).

Conclusion: When prescribing meldonium to patients with stable angina, one should take into account, the genetic information on the individual polymorphism of the CYP2C9 gene and give preference to individuals who have the pathologic genotype of CYP2C9*2 C430T (CT) in view of the predicted genoprotective effect of the drug.

KEY WORDS: Genoprotection, Genotoxicity, Meldonium, Personalized pharmacotherapy, Pharmacogenetics, Polymorphism of the gene CYP2C9*2 C430T

INTRODUCTION

The introduction of the principles of personalized medicine into practical public health in the Russian Federation is declared as one of the priority strategic directions of Russia's scientific and technological development for the next 10–15 years.[1] Today, the main base of drugs prescribing personalization is considered the knowledge about the genetic polymorphism of drugs biotransformation system.[2] One of such clinically significant enzymes of biotransformation is cytochrome CYP2C9, which is a protein with a molecular weight of 55 k Dalton, consisting of 490 amino acid residues. The gene of this enzyme is located in the locus 10q24.1.24.3 of the 10th chromosome.[3] Cytochrome CYP2C9 is synthesized in liver cells and participates in biotransformation of 10% of therapeutically important drugs such as anticoagulant warfarin, anticonvulsant phenytoin, antidiabetic drug tolbutamide, non-

Access this article online

Website: jprsolutions.info | ISSN: 0974-6943

[1]Department of Medicine, *Belgorod State* National Research *University*, *Belgorod*, Russia, [2]Department of Medicine, Voronezh State Medical University Named after N. Burdenko, Voronezh, Russia

***Corresponding author:** Olesya V. Romaschenko, *Belgorod State* National Research *University*, 85, Pobedy Street, *Belgorod* - 308015, Russia. E-mail: Romashenko@bsu.edu.ru

Received on: 28-09-2017; Revised on: 11-10-2017; Accepted on: 01-12-2017

steroidal anti-inflammatory drugs (ibuprofen, diclofenac, etc.), and losartan.[4-6] The pathological gene of cytochrome CYP2C9*2 causes the synthesis of the enzyme with altered catalytic activity (only 12% of the activity of normal CYP2C9).[2] Therefore, carriers of the variant CUR2C9*2 are considered as "slow" metabolizers in both homozygous and heterozygous states: They have a reduced metabolism of drugs, which accumulate in high concentrations in the body, which can lead to undesirable drug reactions, including intoxication.[7] Along with the function of biotransformation of xenobiotics, cytochrome CYP2C9 also has the function of synthesizing from the polyunsaturated fatty acids of cell membranes of eicosanoids - local action hormones, highly active mediators, which take part in various processes, in particular, in hemostasis and vasodilation.[8] The subject of our study was a preparation of the metabolic type of action meldonium, which has an additional endothelioprotective effect due to the stimulation of nitric oxide production, which is important for patients with ischemic heart disease (IHD). Whether polymorphism of cytochrome CYP2C9 gene is important for the development of cytoprotective properties of meldonium is not described in the literature.

The purpose of this study was to study the polymorphism of cytochrome CYP2C9 gene and to assess the significance of this factor in the manifestation of the genoprotective properties of meldonium in patients with stable angina.

MATERIALS AND METHODS

A total of 90 patients with IHD: Stable angina pectoris was examined: 63 men and 27 women aged from 37 to 81 years (mean age of patients was 59.26 ± 0.74 years). Clinical examination of patients in the initial status was carried out when they entered the Cardiology Departments of the Belgorod Regional Clinical Hospital of St. Joasaph. Each participant was acquainted with the research program and signed informed consent. In the majority of patients, angina pectoris was associated with hypertension-80 (89.4%), rhythm disorders-22 (24.4%), postinfarction cardiosclerosis-44 (48.8%), chronic heart failure-85 (94.4%), and in some with Type II diabetes-21 (23.1%). The diagnosis of IHD: Stable angina pectoris was verified after clinical, instrumental, and laboratory examination in accordance with the recommendations of the European Society of Cardiology (ESC).[9]

The program of examination of patients included the implementation of general clinical methods of investigation, instrumental, and laboratory, including electrocardiography, echodoplercardiography, coronary angiography, treadmill test, general and biochemical blood tests with the determination of coagulogram, glucose, potassium, creatinine, and other parameters according to the recommendations of the ESC.[9]

Gene polymorphisms of CYP2C9*2 C430T were determined by polymerase chain reaction using presets reagents of firm "Liteh" (Russia) in the Center of genomic selection of Belgorod State University. DNA was isolated from blood leukocytes from patients.

The potential genotoxicity or genoprotective effect of meldonium was studied by the method of DNA comet assay by testing the drug *in vitro* on the leukocytes of patients' blood according to the method developed by us.[10] The DNA comet index and the index of its growth, % of DNA in the tail, were determined by the method of DNA comet assay.[11] Studies using the method of DNA comet assay were carried out in the Center of genomic selection of the Belgorod State University.

In the summary tables, only reliable data were entered for further analysis. The statistical processing of the material was carried out by the method of variational statistics. The difference between the two groups was assessed according to Student's *t*-test. The results were considered statistically significant at $P < 0.05$. The criterion χ^2 was used to estimate the correspondence of the sample distribution to predetermined distributions (the Hardy–Weinberg law). To evaluate the results of the DNA comet assay method, comet assay software was used. During the calculations, the programs "Microsoft Excel 2007" and "SPSS for Windows 11.0" were used.

RESULTS AND DISCUSSION

When analyzing the CYP2C9*2 C430T gene, 75 people (83%) were found to be homozygous for the normal allele (CC genotype), and 15 people (17%) were heterozygous (genotype CT), i.e., together with the normal allele are pathological one [Figure 1]. Homozygous people with the pathological allele (TT genotype) were not found in the sample. Thus, the frequency of occurrence of the pathological allele is 0.092, and the normal one is 0.908. There is a small excess of heterozygotes (coefficient of inbreeding $F = -0.102$), as well as a low value of the index of genetic variability of the Shannon index ($I = 0.308$). However, in this case, the genotypic structure of the sample under study is generally consistent with the Hardy–Weinberg law ($\chi^2 = 0.953$, $P = 0.329$, $Df = 1$), which allows us to speak about the relative stability of the population for a given gene.

A comparative analysis was performed between groups of patients with the normal and pathological

gene CYP2C9*2 C430T. A number of significant differences were found [Tables 1 and 2]. In the carriers of the pathological cytochrome CYP2C9*2 C430T gene (CT genotype), the following features were observed: A higher functional class of angina, excess body weight, a tendency to hypercoagulation of blood (despite the fact that all patients took antiplatelet agents), a significant relationship with the psychological profile increased the level of personal anxiety, and the DNA in the original status is more damaged.

Carriers of the pathological cytochrome CYP2C9*2 gene C430T (CT genotype) were found out the ability of meldonium to have a genoprotective effect, whereas in patients with the CC genotype mildonium exhibited genotoxic properties [Figure 2].

As mentioned above, cytochrome CYP2C9 has the function of biotransformation of xenobiotics and the function of synthesizing from the polyunsaturated fatty acids of cell membranes of eicosanoids - local action hormones, highly active mediators, which take part in various processes, in particular, in hemostasis and vasodilation.[8] The biochemistry of the reaction is as follows: Under the action of phospholipase, A2 from phospholipids of endothelial cell membranes, arachidonic acid is released, which in three ways are converted into various eicosanoids - prostaglandins under the action of cyclooxygenase, leukotrienes by lipoxygenase and epoxyeicosatrienoic acids (EETs) by cytochromes through the P450 monooxygenase pathway. These EETs reduce blood clotting, reduce platelet aggregation, produce vasodilation, stimulate angiogenesis, have an anti-inflammatory effect, and also protect the heart from "damage" in ischemia-reperfusion. EETs produce these functional effects by activating receptor-mediated signaling pathways and ion channels.[8]

The presence of the pathological CYP2C9* 2 gene in humans causes the synthesis of the so-called "slow" enzyme of cytochrome 2C9, which in turn leads not only to a decrease in the biotransformation rate of drugs, as mentioned above,[7] but also to a violation of eicosanoid synthesis, which can lead to increased blood clotting, increased platelet aggregation, vasoconstriction, and proinflammatory effects (excessive formation of free radicals of oxygen). The phenomenon of association of the presence of the pathological gene CYP2C9*2 with a higher functional class of angina in patients with IHD, the tendency of blood to hypercoagulability, greater DNA damage, can be explained by a significant decrease in the synthesis of eicosanoids-epoxyeicosatrienoic acids and activation of free-radical oxidation, which can destroy DNA.

Excess body weight contributes to stagnation of blood and its thickening, increased personal anxiety is a known risk factor for cardiovascular disease.[12,13] In other words, according to our study, the reduced activity of cytochrome CYP2C9 observed in carriers of the pathological gene CYP2C9*2 C430T is associated with a more severe course of angina pectoris, which is consistent with the literature data.

The phenomenon of manifestation of the genoprotective properties of meldonium in patients with the pathological cytochrome CYP2C9*2 C430T gene and the genotoxicity of meldonium in patients with a normal genotype for this allele, which we

Table 1: Comparative analysis of groups of patients with IHD having a normal and pathological gene CYP2C9*2 C430T

Index	Patients with normal genome CC	Patients with a pathological gene CT	*P*
Weight, kg	85.03±2.07	99.24±4.46	0.002
Quetelet index, kg/m^2	29.90±0.65	33.14±1.37	0.020
Prothrombin index, %	96.29±1.22	100.00±0	0.003
International normalized attitude	1.07±0.03	1.00±0	0.036
DNA: The number of damaged cells, %	3.43±1.13	11.00±3.21	0.020
DNA: Meldonium, an increase in the index of DNA comets	2.72±1.20	0.17±0.29	0.053

The reliability of the differences was assessed by the Student's *t*-test. IHD: Ischemic heart disease

Table 2: Comparative analysis of groups of patients with IHD having a normal and pathological gene CYP2C9*2 C430T

Index	Patients with normal genome	Patients with a pathological gene	*P*
Functional class of angina pectoris	2.33/3.00 (2.00; 3.00)	2.79/3.00 (2.00; 3.00)	0.070
The degree of obesity	0.64/0.00 (0.00; 1.00)	1.24/1.00 (0.00; 2.00)	0.039
Personal anxiety, scores	48.28/47.00 (41.50; 54.50)	53.14/52.50 (47.75; 58.25)	0.061
DNA: The maximum degree of damage	1.60/2.00 (0.50; 2.00)	2.45/2.00 (2.00; 3.00)	0.023

The numerator is the arithmetic mean; the denominator is the median, 25% and 75% quartile. The reliability of the differences was evaluated according to the Mann–Whitney U criterion. IHD: Ischemic heart disease

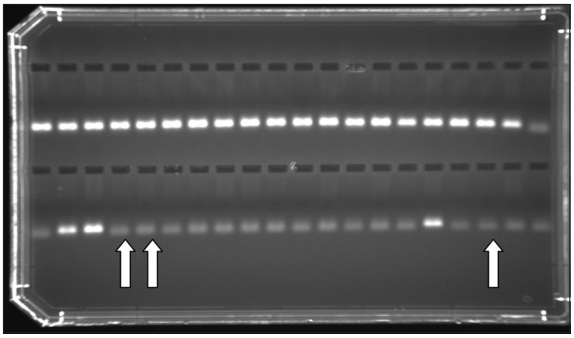

Figure 1: Electrophoregram of polymorphism of the gene CYP2C9*2 C430T in patients with ischemic heart disease. The arrows denote heterozygotes along the pathological allele of the CT. The unmarked positions correspond to homozygotes over the normal CC allele.

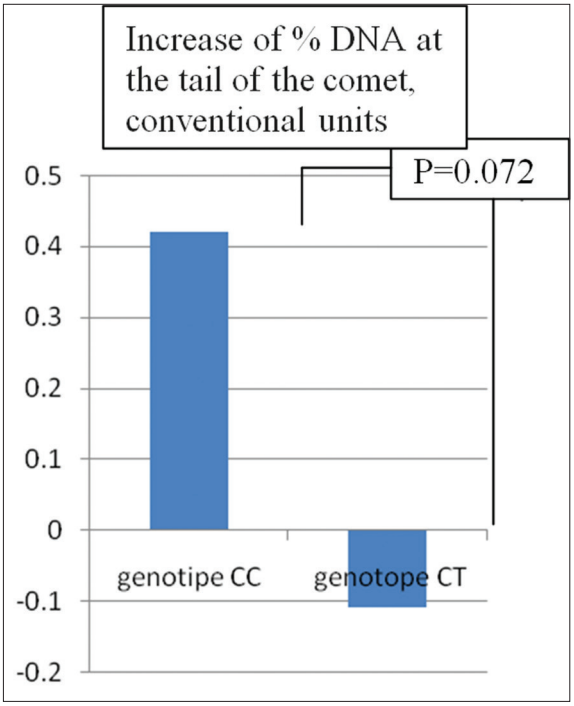

Figure 2: Increase of % DNA at the tail of the comet of blood leukocytes in patients with ischemic heart disease with CC and CT genotypes of cytochrome CYP2C9*2 C430T when injected into the sample meldonium (*in vitro* testing). The reliability of the differences was assessed by Student's *t*-test

discovered, is new, has not been described in the literature.

As a theoretical justification for the phenomenon we discovered, we can give the following. It is known that meldonium blocks the synthesis of carnitine from gamma-butyrobetaine, which decreases the amount of carnitine (the carrier of free fatty acids in the mitochondria), and the metabolic effects of the drug appear, and the amount of gamma-butyrobetaine that irritates the acetylcholine receptors of the endothelium and stimulates the production of nitric oxide what are the endothelioprotective effects of the drug.[14] In experimental studies, the presence of both cardiocytoprotective[15,16] and endothelioprotective properties in melondonium was demonstrated.[17,18] Endothelium of the vessels is the target organ for pharmacological correction of cardiovascular pathology.[19,20] It is likely that the ability of meldonium to have a positive effect on the vascular endothelium and to correct endothelial dysfunction is of value only if there is one. Those, in patients with the pathological gene of cytochrome CYP2C9, due to the "slowing down" of the enzymatic activity of the latter, it is justified to reduce the synthesis of EETs with the corresponding effects of increasing blood clotting, endothelial dysfunction, vasoconstriction, and proinflammatory reactions. In such situation, the introduction of meldonium can have a positive effect due to the accumulation of nitric oxide, which possesses antiaggregant, endothelioprotective, and anti-inflammatory effects at the level of the vessel wall. At the same time, the normal fermentative activity of cytochrome with normal EET synthesis is expected in patients with a normal CYP2C9 genotype and with corresponding manifestations of antiaggregant, endothelioprotective, and anti-inflammatory effects of the latter. In such a situation, the introduction of meldonium can unbalance the balanced system of eicosanoids, up to the damage at the DNA level, since the excessive activity of nitric oxide can cause accumulation of peroxynitrite and oxidative stress.

SUMMARY

Thus, the genetic factor is essential for the pathogenesis of the development of IHD, which determines the ability of meldonium as a metabolic corrector to have a genoprotective or genotoxic effect, depending on the polymorphism of the cytochrome CYP2C9 gene, which must be taken into account in the personalized appointment of the latter in clinical practice.

CONCLUSIONS

1. To realize the pharmacodynamic effects of the metabolic corrector of meldonium and a personalized approach to its use in patients with IHD, the genetic factor, namely, the polymorphism of the gene of the biotransformation system of drugs - CYP2C9*2 C430T, can be important.
2. In patients with IHD having a pathological cytochrome CYP2C9*2 C430T gene (CT genotype), in comparison with patients with normal genotype for this allele (CC), a higher functional class of angina pectoris, overweight, blood hypercoagulability, an increased level of personal anxiety, and greater DNA damage were revealed.
3. In patients with IHD having a pathological cytochrome CYP2C9*2 C430T gene (genotype CT) was found out the ability of meldonium to have a genoprotective effect, whereas in patients with a normal CC genotype meldonium exhibited genotoxic properties.

REFERENCES

1. Putin VV. Ukaz Prezidenta RF ot 1 Dekabrya 2016 g 642 O Strategii Nauchno-Tekhnologicheskogo Razvitiya Rossiyskoy Federatsii; 2016. Available from: http://www.garant.ru/products/ipo/prime/doc/71451998/#ixzz4i6FhzqgF. [Last accessed on 2017 Jul 05].
2. Sychev DA, Ramenskaya GV, Ignatiev IV, Kukes VG. In: Kukes VG, Bochkova NP, editors. Clinical Pharmacogenetics: Textbook. Moscow: GEOTAR-Media; 2007. p. 248.
3. Maharin OA. The Distribution of the Genotypes CYP1A1 (Ile462Val), CYP2C9* 2, CYP2B6* 2, CYP2B6* 6, CYP3A4* 1B Among Residents of Rostov-on-Don. Living and Biosidic Systems; 2012. p. 1. Available from: http://www.jbks.ru/archive/issue-1/article-9. [Last accessed on 2017 Sep 2017].
4. Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, Goldstein JA, *et al.* Tolbutamide, flurbiprofen, and losartan as probes of CYP2C9 activity in humans. J Clin Pharmacol 2003;43:84-91.
5. Rettie AE, Jones JP. Clinical and toxicological relevance of CYP2C9: Drug-drug interactions and pharmacogenetics. Annu Rev Pharmacol Toxicol 2005;45:477-94.
6. Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: A HuGEnet systematic review and meta-analysis. Genet Med 2005;7:97-104.
7. Korchagina RP, Osipova LP, Vavilova NA, Voronina EN, Filippenko ML. Genetic polymorphism of cytochrome P450 2C9, involved in the metabolism of drugs in indigenous populations of the North Siberia. Bull SB RAMS 2011;31:39-44.
8. Spector AA, Kim HY. Cytochrome P450 epoxygenase pathway of polyunsaturated fatty acid metabolism. Biochim Biophys Acta 2015;1851:356-65.
9. Montalescot G, Sechtem U, Achenbach S. Guidelines on the management of stable angina pectoris: Executive summary: The task force on the management of stable angina pectoris of the European Society of Cardiology. Eur Heart J 2013;34:2949-3003.
10. Snegin EA, Romaschenko OV, Ye SN. A Method for Predicting the Individual Efficacy and Safety of Metabolic Drugs in Influencing the Human Genome in *in vitro* Samples. Certificate No. 90 on Registration as a know-how of the Result of Intellectual Activity. Belgorod: NIU, BelGU; 2012.
11. Collins AR, Oscoz AA, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, *et al.* The comet assay: Topical issues. Mutagenesis 2008;23:143-51.
12. Psheninnikova MG. The phenomenon of stress. Emotional stress and its role in pathology. Pathol Physiol Exp Ther 2000;4:21-31.
13. Psheninnikova MG. The phenomenon of stress. Emotional stress and its role in pathology (continued). Pathol Physiol Exp Ther 2001;1:26-31.
14. Ya KI. Synthesis and biological activity of the new bioregulator-mildronate. Experimental and clinical pharmacotherapy. Riga Zanatne 1991;19:7-14.
15. Danilenko LM, Pokrovskii MV. 3-(2,2,2-trimethylhydrazinium) propionate: New concept of realization of cardioprotective effect. Res J Pharm Biol Chem Sci 2014;5:1419-22.
16. Danilenko LM, Klochkova GN, Kizilova IV, Korokin MV. Metabolic cardioprotection: New concepts in implementation of cardioprotective effects of meldonium. Res Result Pharmacol Clin Pharmacol 2016;2:95-100.
17. Korokin MV, Pashin EN, Bobrakov KE, Pokrovskii MV, Ragulina VA, Artyushkova EB, *et al*. The study of the endothelium protective and coronary actions of 3-oksipiridin derivatives. Kuban Sci Med Bull 2009;4:104-8.
18. Skachilova SY, Kesarev OG, Danilenko LM, Bystrova NA, Dolzhikov AA, Nikolaev SB. Pharmacological correction of L-NAME-induced oxide deficiency with derivatives of 3-(2,2,2-trimethylhydrazinium) propionate. Res Result Pharmacol Clin Pharmacol 2016;1:36-41.
19. Molchanova OV, Pokrovskaya TG, Povetkin SV, Reznikov KM. Endothelioprotective property of the combination of the thioctic acid and rosuvastatin shown in the endothelial dysfunction models. Res Result Pharmacol Clin Pharmacol 2016;2:9-15.
20. Ragulina VA, Kostina DA, Dovgan AP, Burda YE, Nadezhdin SV. Nuclear factor kappa B as a potential target for pharmacological correction endothelium-associated pathology. Res Result Pharmacol Clin Pharmacol 2017;3:114-24.