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Research Article

**GENE POLYMORPHISM OF ENDOTHELIAL NITRIC OXIDE  
SYNTHASE IN PATIENTS WITH STABLE ANGINA PECTORIS  
AND ITS SIGNIFICANCE IN THE MANIFESTATION OF  
GENOPROTECTIVE PROPERTIES OF MELDONIUM****Olesya V. Romaschenko<sup>1\*</sup>, Eduard A. Snegin<sup>1</sup>, Lyudmila R. Zakirova<sup>1</sup>, Elena B. Artyushkova<sup>2</sup>, Galina A. Batischeva<sup>2</sup>, Natalya A. Bystrova<sup>2</sup>, Vladimir Y. Provotorov<sup>2</sup>**<sup>1</sup>Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia<sup>2</sup>Kursk State Medical University, 3, K. Marx St., Kursk, 305041, Russia**Abstract**

**Introduction:** Personalized pharmacotherapy of stable angina pectoris implies an in-depth study of the pharmacogenetic factor when prescribing the recommended drugs in order to increase the effectiveness and safety of their use. The work is devoted to the development of individual approaches to the appointment of meldonium in the complex pharmacotherapy of angina pectoris as a cardiac cytoprotector with endothelioprotective properties, depending on gene polymorphism of endothelial nitric oxide synthase.

**Research tasks:** To study the polymorphism of the endothelial nitric oxide synthase gene eNOS C786T and to evaluate the significance of this factor in the manifestation of the genoprotective properties of meldonium in patients with stable angina pectoris.

**Methods:** A total of 90 patients with stable angina pectoris were examined. DNA was isolated from blood leukocytes from patients. The polymorphism of endothelial nitric oxide synthase eNOS C786T was determined by polymerase chain reaction. 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. The content of cortisol, cyclic AMP and cyclic GMP in the blood serum of patients, concentration of endothelial and inducible nitric oxide synthases in erythrocytes was determined by the enzyme immunoassay.

**Results:** The presence of gene polymorphisms of eNOS C786T alleles was found. They revealed a more severe clinical condition of patients having pathological genes by eNOS C786T alleles in the form of signs of a decrease in the activity of stress-limiting and increasing activity of stress-realizing systems at the level of humoral regulation and intracellular messengers in connection with the apparent decrease in the enzymatic activity of endothelial nitric oxide synthase. Potential genotoxicity of meldonium was revealed when it was administered to patients with a pathological genotype of endothelial nitric oxide synthase eNOS C786T (CC, CT) and a genoprotective effect when administered to patients with a normal genotype of endothelial nitric oxide synthase eNOS C786T (TT).

**Conclusion:** When prescribing meldonium to patients with stable angina, one should take into account the genetic information on the individual polymorphism of the endothelial nitric oxide synthase gene and give preference to individuals who have the normal genotype of eNOS C786T (TT) in view of the predicted genoprotective effect of the drug.

**Key words:** pharmacogenetics, polymorphism of the gene eNOS C786T, meldonium, genotoxicity, genoprotection, personalized pharmacotherapy.

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**INTRODUCTION:**

Genetics occupies a dominant position in the developing direction of personalized medicine today [1, 2]. This is primarily due to achievements in fundamental human genetics, with the decoding of the genome and the end in 2003 of the international program "Human Genome", which allowed a number of authors to regard the 21st century as a century of genetics [1]. Abroad (in countries of Western Europe and the USA), where the human genome is decoded with the help of high technologies, work is actively being done towards the creation of a genetic passport, personalized medicine services are being organized with computer software and business financing projects [3, 4].

One of the most promising areas of personalized medicine is pharmacogenetics, a science that studies the role of genetic factors in the formation of the pharmacological response of the human body to medicines [5]. Genotyping methods make it possible to predict the pharmacological response to medicines, which can significantly improve the effectiveness and safety of the use of medications [6]. If a corresponding allele variant is found, the patient needs correction of therapy (dose, route of administration and its multiplicity, replacement of the drug, etc.). The study of genes responsible for the pharmacokinetics and pharmacodynamics of drugs is widely introduced into clinical practice in all developed countries, and is the subject of clinical studies [6, 7].

The subject of our study was the preparation of the metabolic series meldonium, which is used in cardiology to increase the effectiveness of the complex treatment of coronary heart disease by providing cardiac cytoprotection [8, 9]. In the mechanism of meldonium action the possibility of additional influence of this preparation on the vascular endothelium is established by regulation of the production of nitric oxide [9]. In experimental studies, the presence of both cardiocytoprotective [10, 11] and endothelioprotective properties in melondonium was demonstrated [12, 13]. Endothelium of the vessels is the target organ for pharmacological correction of cardiovascular pathology [14, 15]. Polymorphism of the endothelial nitric oxide synthase gene can determine the different enzymatic activity of NO synthase and, accordingly, a different baseline level of nitric oxide production [16]. Whether this factor is important for the realization of potentially positive properties of meldonium is not described in the literature.

The purpose of this study was to study the polymorphism of the endothelial nitric oxide synthase gene eNOS C786T 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 stable angina pectoris were examined: 63 men and 27 women aged from 37 to 81 years (mean age of patients was  $59.26 \pm 0.74$  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%), in some - with type II diabetes - 21 (23.1%). The diagnosis of stable angina pectoris was verified after clinical, instrumental, laboratory examination in accordance with the recommendations of the European Society of Cardiology (ESC) (2013) [17].

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 determination of coagulogram, glucose, potassium, creatinine and other parameters according to the recommendations of the ESC (2013) [17]. Key metabolites of functional systems that are responsible for the process of adaptation - stress-realizing (cortisol, cAMP, inducible nitric oxide synthase) and stress-limiting (cGMP, endothelial nitric oxide synthase) systems were studied [18]. The study of hormones, enzymes and intracellular messengers with the opposite regulatory action allows us to judge the state of humoral regulation. The concentration of cortisol, cAMP and cGMP was determined in the blood serum, and the levels of endothelial and inducible nitric oxide synthase in the erythrocyte lysate by the method of immunoenzymatic analysis on the BioRad apparatus using reagents sets of the firm "Biochimmak". The studies were carried out on the basis of the clinical diagnostic laboratory of the Belgorod Regional Clinical Hospital of St. Joasaph. Gene polymorphisms of endothelial NO-synthase eNOS C786T was determined by polymerase chain reaction using presets reagents of firm "Litech" (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

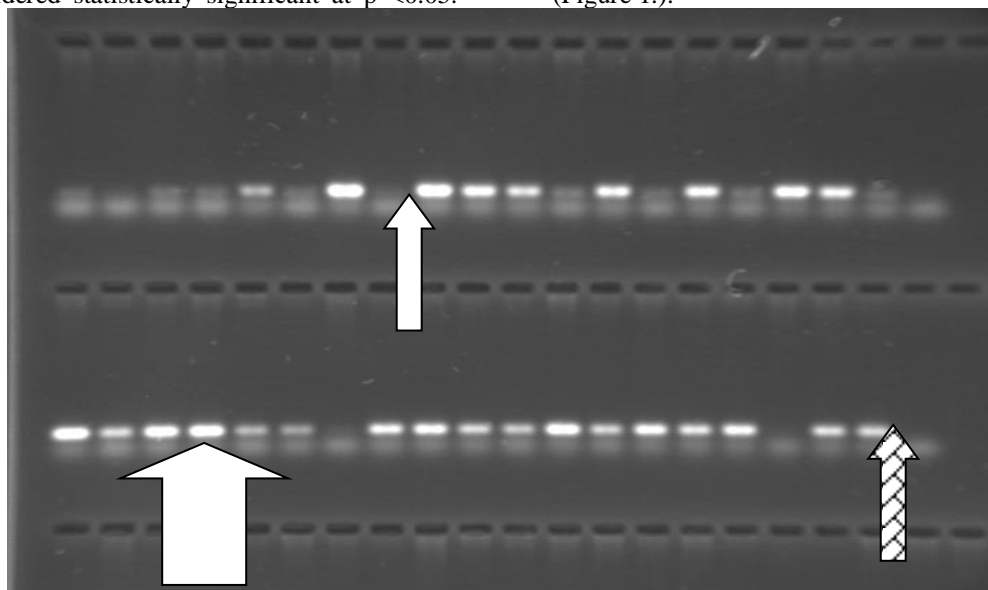
leukocytes of patients' blood according to the method developed by us [19]. The DNA comet index and the index of its growth, % of DNA in the tail were determined by the method of DNA comet assay [20]. Studies using the method of DNA comet assay were carried out in the Center of genomic selection of the 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  $\chi^2$  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, CometAssey software was used. During the calculations, the programs "Microsoft Excel 2007" and "SPSS for Windows 11.0" were used.

### RESULTS AND DISCUSSION:

The study of the polymorphism of the eNOS C786T gene revealed a high degree of variability in the analyzed sample of patients for this genetic marker (Figure 1.).



**Fig 1: Electrophoregram of polymorphism of the gene eNOS C786T in patients with stable angina pectoris**  
**Note. The thick arrow is homozygotes along the normal allele (TT), the thin arrow is homozygous for the pathological allele (CC), the thin shaded arrow is heterozygote (CT).**

**Table 1: Comparative analysis of groups of patients with ischemic heart disease having a normal and pathological nitric oxide synthase gene NOS C786T**

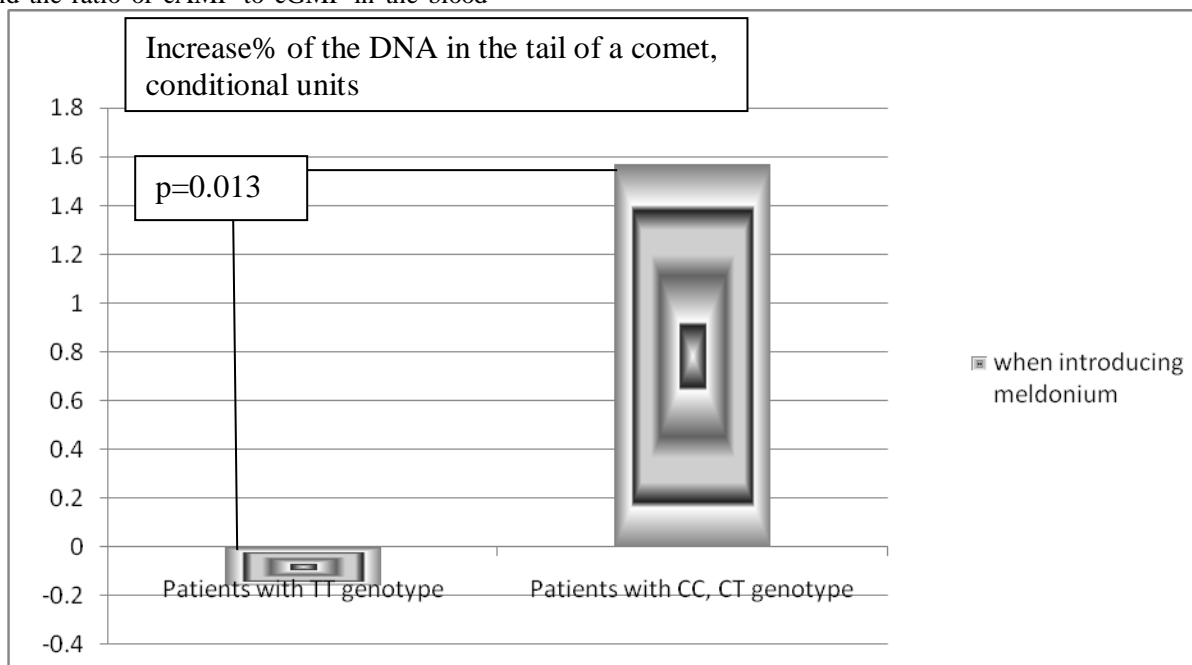
Index	Patients with normal genome (TT)	Patients with a pathological gene (CC, CT)	p
Heart rate, beats / min	67.80±2.27	76.00±2.29	0.026
Blood glucose, mmol / l	5.62±0.20	8.12±0.84	0.008
Blood creatinine, mmol / l	89.34±2.54	99.41±3.46	0.020
Potassium of blood, mmol / l	4.55±0.08	4.89±0.11	0.014
Blood serum cortisol, µg / dL	6.32±0.42	11.63±2.57	0.056
cAMP serum, pmol / ml	0.99±0.24	2.32±0.76	0.066
cAMP / cGMP serum	9.38±3.69	22.83±2.79	0.020
eNOS in the lysate of erythrocytes, ng / ml	1039.23±444.31	574.10±117.22	0.341
iNOS in the lysate of erythrocytes, ng / ml	28.40±3.04	15.81±4.93	0.046
DNA: melatonin: an increase in the index of DNA comets	-0.16±0.19	1.45±0.53	0.030

**Comment:** the reliability of the differences was assessed by the Student's t-test

Carriers of the pathological gene of endothelial nitric oxide synthase eNOS C786T (CC and CT genotypes) observed a number of features: higher heart rate by almost 10 beats per minute (which may negatively affect on future life expectancy), hyperglycemia, higher levels of creatinine and potassium in blood (it is possible to connect with the functional activity of the kidneys), almost 2 times decreased level of endothelial and inducible nitric oxide synthases in the erythrocyte lysate, increased level of cortisol, cyclic AMP and the ratio of cAMP to cGMP in the blood

serum (as a reflection of the decrease in activity of stress-limiting and increasing activity of stress-realizing systems at the level of humoral regulation and intracellular messengers).

The introduction of meldonium in vitro to patients with the pathological eNOS gene was accompanied by negative effects in the form of destruction of blood leukocyte DNA, and in patients with the normal eNOS genotype - positive effects in the form of restoration of destroyed DNA (Figure 2.).



**Fig 2: Increase in % of DNA in the tail of a comet of blood leukocytes in patients with angina pectoris with genotypes of TT and CC, CT of endothelial nitric oxide synthase eNOS C786T when injected into the sample meldonium (in vitro testing)**

Note. The reliability of the differences was assessed by Student's t-test.

Endothelial nitric oxide synthase eNOS is an enzyme responsible for the synthesis in the vascular wall of NO - the most important vasodilator, antiaggregant, antimitogen and antioxidant [21]. In vascular endothelium, NO plays a key role in relaxation and reduction of migration and proliferation of vascular smooth muscle cells, inhibition of adherence of platelets and leukocytes to the endothelium, inhibition of oxidation of low density lipoproteins [22]. The versatility of the functions of nitric oxide as an endothelial factor of vasodilation and as a mediator of the NO-stress-limiting system is widely described in the literature and agrees with the results of our study [23].

The endothelial nitric oxide synthase gene is localized in the 7th chromosome [24]. The mutation

in the polymorphic region of the eNOS C786T gene is associated with a decrease in NO production [16] and may cause coronary artery spasm [25].

The data obtained by us, firstly, show a direct correlation between the pathology of the eNOS C786T gene and the decrease in the production of nitric oxide synthases (not only endothelial, but also inducible); Secondly, they demonstrate the association of this phenomenon with the deterioration in the clinical status of patients with angina pectoris (increased heart rate, increased glucose, creatinine, potassium in the blood), and third, reflect the processes of activation of stress-realizing systems in patients with angina (in terms of increasing cortisol, cAMP, cAMP/cGMP in the blood) under conditions of insufficient synthesis of nitric oxide.

The ability of meldonium to demonstrate genotoxic properties in patients with ischemic heart disease having a pathological endothelial nitric oxide synthase gene, eNOS C786T, and genoprotective properties under the normal genotype of eNOS C786T, is a new one. Obviously, the presence of endothelial dysfunction due to a decrease in the activity of endothelial nitric oxide synthase in patients with the pathological eNOS C786T gene, with an appropriate restriction of the possibility of NO production, predetermines the genotoxicity of the drug whose mechanism of action is associated with endothelial function. 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 [9].

Since the cytoprotective effectiveness of meldonium is dependent on the functional state of the endothelium, it is logical to explain the effect of this metabolic corrector on the DNA of cells, depending on the genotype of eNOS, presented in our study.

### CONCLUSION:

Thus, when prescribing meldonium to patients with stable angina pectoris, one should take into account the genetic information on the individual polymorphism of the endothelial nitric oxide synthase gene and give preference to individuals who have the normal genotype of eNOS C786T (TT) in view of the predicted genoprotective effect of the drug.

### OUTPUTS

1. To realize the pharmacodynamic effects of the metabolic corrector of meldonium and its personalized choice when assigned to patients with coronary heart disease, the genetic factor, namely the polymorphism of the endothelial nitric oxide synthase gene eNOS C786T, is important.

2. In patients with angina pectoris who have a pathological endothelial nitric oxide synthase gene, eNOS C786T (CC and CT genotypes), a number of features have been found in comparison with those with normal genotype for this allele (TT): higher heart rate, hyperglycemia, higher creatinine and potassium levels in the blood, a reduced level of endothelial and inducible nitric oxide synthases in the lysate of erythrocytes, elevated levels of cortisol, cyclic AMP, and the ratio of cAMP to cGMP in serum.

3. Meldonium is able to exhibit genotoxic properties in patients with angina pectoris who have a

pathological endothelial nitric oxide synthase gene, eNOS C786T (CC, CT) and genoprotective properties in individuals with the normal eNOS C786T (TT) genotype.

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