

## Age-related features of the pathogenesis of ischemic heart disease and sensitivity of patients' DNA to cytoprotectors

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### ABSTRACT

**Introduction:** The high prevalence of coronary heart disease (CHD) and the increase in mortality from this nosology among patients of older age groups determine the urgency of the problem of improving the provision of medical care to this category of patients. This research is devoted to the study of the effect of cytoprotective therapy on the course of coronary artery disease in elderly patients in terms of its necessity and feasibility. **Objective:** The objective of the study was to study the age-related features of the pathogenesis of CHD and the sensitivity of patients' DNA to cytoprotectors: Meldonium, phosphocreatine, and cytoflavin. **Methods:** A randomized clinical trial was conducted in 160 patients with coronary artery disease: Stable exertional angina. The patients were divided into two groups depending on their age: The Group 1 consisted of 82 patients of middle age (from 45 to 60 years old, on average  $51.56 \pm 0.61$  years); the Group 2 consisted of 78 elderly patients (from 60 to 75 years old, on average  $66.76 \pm 0.57$  years). All patients underwent coronary angiography, blood test for adenosine triphosphate (ATP) content in serum by spectrophotometric method. DNA status of blood leukocytes of patients in response to the introduction of metabolic correctors (meldonium, phosphocreatine, and cytoflavin) *in vitro* was assessed by the comet assay. **Results:** The age-related factor has a significant effect on the pathogenesis of CHD: In the elderly, involutive hypoergosis is observed (a decrease in the serum ATP content to  $207.20 \pm 3.52$   $\mu\text{mol/l}$  compared to patients of middle age  $227.39 \pm 4.34$   $\mu\text{mol/l}$ ,  $P = 0.001$ ), a more pronounced atherosclerotic impairment of the coronary vessels with the involvement of at least 4 vessels with an average degree of stenosis from 23% to 64% ( $41.09 \pm 2.75\%$ ), a longer history of concomitant diabetes mellitus, with greater severity of its course, despite significantly less common risk factors for smoking and obesity. The studied metabolic correctors (meldonium, phosphocreatine, and cytoflavin), according to *in vitro* testing, have a more favorable profile of influence on the DNA of cells in elderly patients compared to middle-aged patients, which indicates the possibility of their use as geroprotectors. **Conclusion:** On the one hand, the identified pathogenetic features of CHD in elderly patients in the form of involutive hypoergosis and more pronounced atherosclerosis of the coronary vessels, necessitate the inclusion of cytoprotectors in the treatment program; on the other hand, the detected geroprotective properties of the studied metabolic correctors determine the feasibility of their use in old age.

**KEY WORDS:** Cytoprotectors, DNA, Geroprotection, Ischemic heart disease, Old age

### INTRODUCTION

Ischemic heart disease is a worldwide disease, the main cause of death in elderly and senile patients.<sup>[1]</sup> With age, the risk of cardiovascular complications increases significantly, which is reflected in the Systematic Coronary Risk Evaluation scale, where this factor is one of the keys.<sup>[2]</sup> Perhaps this is due to the stress-age syndrome, which occurs in the aging process and causes the disease to worsen.<sup>[3]</sup> It is known that the aging process is accompanied by the destruction

of cellular structures - membranes, mitochondria, DNA, which limits the adaptive capacity of the body, contributes to the development of age-related pathology, and increases the likelihood of death.<sup>[3-5]</sup>

The accepted standards of medical and surgical treatment cannot fully solve the problem. An attempt to significantly increase the effectiveness of treatment is the introduction into clinical practice of cytoprotective therapy aimed at protecting myocardial cells from ischemic damage.<sup>[6]</sup> Cytoprotectors have a preconditioning effect and can increase cell viability under hypoxic conditions.<sup>[7]</sup> Means of metabolic therapy are recommended for use in patients of older age groups as geroprotectors,<sup>[8]</sup> they reveal their

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positive effect when administered in the combination treatment of IHD and CHF in elderly and senile patients.<sup>[9]</sup>

Perhaps the use of cytoprotectors in old age has double feasibility - protects the cells of the heart from ischemic damage and slows down the aging process.

It is of interest to study cytoprotectors with different metabolic orientations: Meldonium as a drug that switches myocardial energy exchange from the consumption of fatty acids to the extraction of energy from carbohydrates; phosphocreatine as a drug that activates the process of substrate phosphorylation, and cytoflavin as a combination drug that activates the process of aerobic glycolysis.

The degree of cell DNA destruction can be classically estimated by the comet assay.<sup>[10]</sup> In our previous works, we showed that cytoprotectors have a certain effect on the DNA of blood leukocytes in patients with coronary artery disease.<sup>[11]</sup> Whether there are age-related features of DNA, sensitivity to cytoprotectors is the topic of the present study.

The objective of this research was to study the age-related features of the pathogenesis of coronary heart disease (CHD) and the sensitivity of patients' DNA to cytoprotectors: Meldonium, phosphocreatine, and cytoflavin.

## MATERIALS AND METHODS

To achieve the objective of the study, we conducted a randomized, open controlled clinical study of 160 patients with coronary artery disease: Stable exertional angina (117 men and 43 women). Patients were divided into two groups depending on age: The Group 1 consisted of 82 patients of middle age (from 45 to 60 years, on average  $51.56 \pm 0.61$  years) and Group 2 - 78 elderly patients (from 60 to 75 years old, on average  $66.76 \pm 0.57$  years). The clinical examination of patients in the original status took place on their admission in the Department of Cardiology №1 of St. Joasaph Belgorod Regional Clinical Hospital. Each participant was made aware of the research program and signed an informed consent. In the majority of patients, angina was combined with hypertension (89.4%), rhythm disturbances (24.4%), post-infarction cardiosclerosis (48.8%), chronic heart failure (94.4%), and in some patients - with type II diabetes mellitus (23.1%). The program of examination of patients included the implementation of general clinical, instrumental and laboratory research methods, including electrocardiography, Doppler echocardiography, coronary angiography (CAG), treadmill test, general, and biochemical blood tests according to the recommendations of the All-Russian Scientific Society of Cardiology and the

European Society of Cardiology (2013).<sup>[6]</sup> In addition, we studied the metabolic profile of serum ATP concentration by the spectrophotometric method,<sup>[12]</sup> the effect of metabolic series drugs (meldonium, phosphocreatine, and cytoflavin) on the cell DNA was evaluated by the comet assay by testing the drugs *in vitro* using the method developed by us.<sup>[13]</sup> A number of indicators were determined using the comet assay: The comet DNA index, the percentage of DNA in the comet tail, the maximum degree of DNA damage (from 1 to 4 points). Studies of the comet assay were performed in the laboratory of population genetics and genotoxicology of the BelsU National Research University.

Only reliable data were entered into the summary tables for further analysis. Statistical processing of the material was performed by the method of variation statistics. Quantitative indicators were evaluated for compliance with the normal distribution using the Kolmogorov–Smirnov test.<sup>[14]</sup> For the indicators that have a distribution close to normal, the arithmetic mean, standard (rms) deviation, and mean error were calculated. For quantitative indicators with a distribution other than normal, as well as for ordinal indicators, the mean, median, 25% and 75% quartiles were calculated. The difference between the two groups was evaluated using the Student's *t*-test, the Mann–Whitney U-test. Results were considered statistically significant at  $P < 0.05$ . We used the  $\chi^2$  criterion to assess the compliance of the sample distribution to predetermined distributions (Hardy-Weinberg law). Comet Assay software was used to evaluate the results of the DNA comets assay. For the calculations, Microsoft Excel 2007 and Statistical Package for the Social Sciences for Windows 11.0 were used.

## RESULTS AND DISCUSSION

A comparative analysis was conducted between two groups of patients of middle and old age. A number of reliable differences were obtained [Tables 1 and 2].

Comparison of patients of different age groups showed the presence of pathogenetic features of CHD in the elderly. Elderly patients have a decrease in height and body weight, a longer history of concomitant diabetes, with a greater degree of severity. Elderly patients smoke 5 times less, however, they have significantly more pronounced atherosclerosis of the coronary vessels and aorta: According to CAG, the average degree of stenosis of almost all vascular heart territories is twice as high in the elderly ( $41.09 \pm 2.75\%$ ), and the number of affected coronary arteries is higher, and averages 4 vessels. In elderly patients, significantly lower serum ATP is observed, which indicates the presence of involutive

hyperergosis due to the development of mitochondrial dysfunction in the aging process.<sup>[14]</sup>

We revealed the unequal sensitivity of the genetic apparatus of the cells of patients of different age groups to the introduction of metabolic correctors, as shown in Figure 1 and Table 2. Thus, the introduction of meldonium, phosphocreatine, and cytoflavin into the sample with a leukocyte suspension *in vitro* in elderly

patients is accompanied by the appearance of DNA in the tail of the comets in a significantly smaller number compared with patients of middle age, which indicates a more favorable effect of metabolic correctors on the genetic apparatus of the cell in elderly patients.

The data obtained may indicate the presence of the geroprotective properties of the drugs we studied, and this does not depend on their metabolic orientation.

**Table 1: A comparative analysis of indicators of pathogenetic features of CHD in patients of middle and old age**

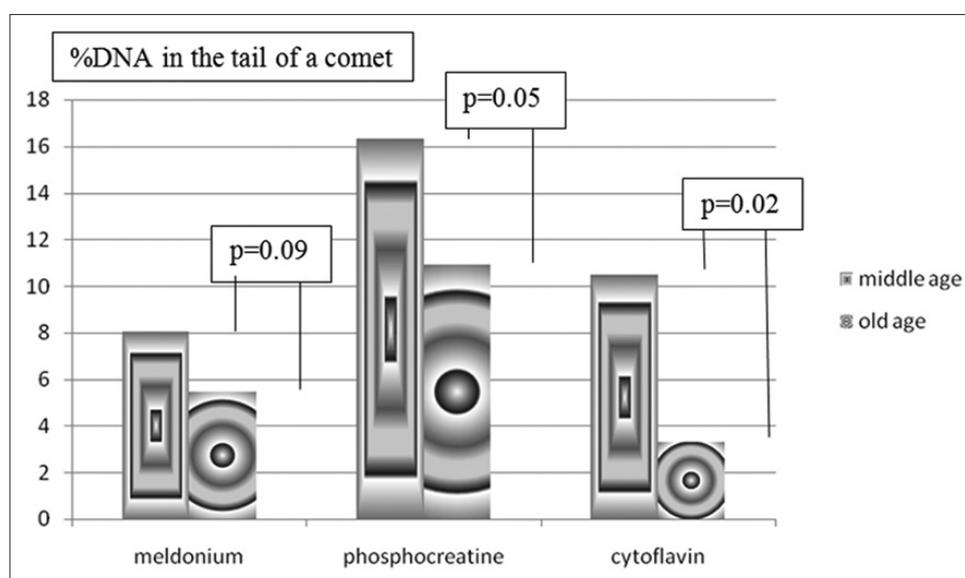
Indicator	Middle-age patients, n=82	Old-age patients, n=78	P
Average age, years	51.56±0.61	66.76±0.57	0.001
Height, m	1.71±0.01	1.68±0.01	0.032
Weight, kg	93.46±2.39	84.33±1.60	0.002
DM: Duration in years	0.72±0.29	2.11±0.55	0.029
Smoking: Number of cigarettes per day	10.35±1.46	2.84±0.65	0.001
Quetelet index, kg/m <sup>2</sup>	31.87±0.76	29.81±0.51	0.026
EchoCG: AV pressure gradient, mm Hg.	6.39±0.41	8.91±0.69	0.002
EchoCG: AV regurgitation	0.51±0.07	0.81±0.10	0.015
CAG: The degree of stenosis of the LCA trunk, %	13.33±3.24	23.11±4.29	0.072
CAG: The degree of the AIVA stenosis, %	51.00±5.03	64.33±4.08	0.042
CAG: The degree of the CA stenosis, %	31.67±4.42	58.00±5.47	0.001
CAG: The degree of the DB stenosis, %	25.00±4.68	47.11±5.09	0.002
CAG: The degree of the AI stenosis, %	6.80±2.61	23.89±5.39	0.005
CAG: The degree of the RCA stenosis, %	38.53±5.37	55.33±5.58	0.033
CAG: Moderate stenosis, %	24.40±2.44	41.09±2.75	0.001
CAG: Number of affected arteries	3.04±0.25	4.33±0.19	0.001
ATP serum, μmol/l	227.39±4.34	207.20±3.52	0.001

The significance of differences was assessed by Student's *t*-test. DM: Diabetes mellitus, AV: Aortic valve, LCA: Left coronary artery, AIVA: Anterior interventricular artery, CA: Circumflex artery, DB: Diagonal branch, AI: Artery intermedia, RCA: Right coronary artery. EchoCG: Echocardiography

**Table 2: A comparative analysis of indicators of pathogenetic features of CHD in patients of middle and old age**

Indicator	Middle-age patients, n=82	Old-age patients, n=78	P
DM: Severity	0.30/0.00 (0.00;0.00)	0.55/0.00 (0.00;0.88)	0.079
DNA: DNA comet index	0.33/0.14 (0.02;0.50)	0.16/0.03 (0.00;0.10)	0.022
DNA: Meldonium: Maximum damage	2.63/3.00 (2.00;4.00)	1.86/2.00 (1.00;3.00)	0.032
DNA: Phosphocreatine: DNA comet index	0.76/0.40 (0.04;0.98)	0.30/0.08 (0.00;0.42)	0.028

The numerator is the arithmetic average, the denominator is the median, 25% and 75% quartile. The significance of differences was assessed by the Mann-Whitney U-test



**Figure 1:** The percentage of DNA in the tail of a comet of blood leukocytes in patients with coronary artery disease on introduction of metabolic correctors into the sample (*in vitro* testing). The differences significance was assessed by student *t*-test

Considering the age-related features of the pathogenesis of coronary artery disease such as a more severe course and involutive hypoergosis in elderly patients, we should say that this category of patients, first, has a higher need for cytoprotectants, and second, the use of the latter in older age groups is of particular expediency due to their geroprotective properties.

## CONCLUSION

The age-related factor has a significant effect on the pathogenesis of CHD: In the elderly, involutive hypoergosis is observed, a more pronounced atherosclerotic impairment of the coronary vessels with the involvement of at least 4 vessels with an average degree of stenosis from 23% to 64% ( $41.09 \pm 2.75\%$ ), a longer history of concomitant diabetes mellitus, with greater severity of its course, despite significantly less common risk factors for smoking and obesity. The studied metabolic correctors (meldonium, phosphocreatine, and cytoflavin), according to *in vitro* testing, have a more favorable profile of influence on the DNA of cells in elderly patients compared to middle-aged patients, which indicates the possibility of their use as geroprotectors.<sup>[15]</sup>

## SUMMARY

1. The age-related factor has a significant effect on the pathogenesis of CHD: In the elderly, involutive hypoergosis is observed, a more pronounced atherosclerotic impairment of the coronary vessels with the involvement of at least 4 vessels with an average degree of stenosis from 23% to 64% ( $41.09 \pm 2.75\%$ ), a longer history of concomitant diabetes mellitus, with greater severity of its course, despite significantly less common risk factors for smoking and obesity.
2. The studied metabolic correctors (meldonium, phosphocreatine, and cytoflavin), according to *in vitro* testing, have a more favorable profile of influence on the DNA of cells in elderly patients compared to middle-aged patients, which indicates the possibility of their use as geroprotectors.

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