The cytoprotective property of ethoxidol in patients with coronary heart disease

Olesya V. Romaschenko[1], Michail V. Pokrovsky[1], Sergey V. Nadezhdin[1], Vladimir G. Kukes[2], Nina I. Zhernakova[1], Petr K. Alferov[1], Natalia D. Grischenko[1], Ludmila R. Zakirova[1], Anastasia V.Morozova[1], Julia A. Savvina[1]

[1]Belgorod State University, Pobedy St., 85, Belgorod, 308015, Russia

E-mail: Romashenko@bsu.edu.ru

[2]First Moscow State Medical University named by I.M.Sechenov, 119991, Moscow, st. Trubetskaya, 8, bld. 2

Abstract

Introduction: The widespread prevalence and high mortality rate from coronary heart disease (CHD), despite the accepted treatment standards, aim at finding the most rational drug combinations, expanding the range of drugs, and developing personalized approaches to their use.

Research tasks: determination of the cytoprotective properties of the antioxidant drug ethoxidol in patients with CHD.

Material and Methods: We examined 30 patients with CHD: stable angina pectoris of I-III functional classes. To determine the cytoprotective properties of ethoxidol, blood leukocytes of patients were studied in vitro by fluorescence microscopy using an Eclipse Ti-U inverted fluorescence microscope (Nikon, Japan). By staining leukocytes with fluorescent dyes (MitoTracker ™ Red CMXRos, Calcein AM, Ethidium bromide), living and dead cells were determined, mitochondrial fluorescence, and the cell viability index (VI_{cells}) was calculated. The materials were processed statistically.

Results: with the introduction of ethoxidol into a sample with a leukocyte suspension, a significant increase in VI_{cells} by 21% (from 41% to 62%, $p < 0.001$) was observed, which indicates the presence of a cytoprotective property in this drug. A more detailed analysis of the dynamics of the VI_{cells} index showed two variants of changes in cell viability: in 80% of patients VI_{cells} was increased, on average, by 28% (from 36% to 64%, $p < 0.001$) and in 20% of patients VI_{cells} was decreased, on average, by 10% (from 68% to 58%, $p < 0.05$). The groups significantly differed in the initial level of cell viability (36% versus 68%, $p < 0.01$), and in the initial level of mitochondrial fluorescence intensity: in the group of increasing cell viability under the influence of ethoxidol, the initial value of mitochondrial fluorescence was significantly lower (114.17 ± 3.63 relative units) in comparison with a group of decreased viability (144.14 ± 10.81 relative units, $p<0.01$). The value of mitochondrial fluorescence changed in both cases upward, but without achieving the reliability of differences.

Conclusion: the antioxidant drug ethoxidol has cytoprotective properties in patients with CHD. At the same time, there is some variability in changes in the viability of blood cells of patients under the influence of ethoxidol in the form of an increase (in 80% of cases) or a decrease (in 20% of cases) of this indicator, which indicates the need to develop a personalized approach to prescribing this drug to patients with CHD.

Key words: antioxidants (ethoxidol), leukocytes, viability, coronary heart disease, patients, microscopy, in vitro study, personalized pharmacotherapy.

INTRODUCTION

The relevance of this study is due to the widespread prevalence, mortality and high social significance of coronary heart disease (CHD) (Gruzdeva, Khokhlov, & Ilyin, 2020; SK et al., 2020; Zyryanov, Fitilev, Vozzhaev, Shkrebniova, & Klyuev, 2020). The search for rational combinations of drugs is of great importance in clinical medicine (Bontsevich, Filinichenko, Gavrilova, Goncharova, & Myronenko, 2018; Bontsevich et al., 2020; Gavrilova, Bontsevich, Vovk, & Balabanova, 2020; Kontsevaya et al., 2017). The accepted standards for the treatment of angina pectoris with drugs from the groups of antiplatelet agents, anticoagulants, beta-blockers, statins, angiotensin-converting enzyme inhibitors, nitrates, calcium antagonists have a high level of evidence, but do not fully ensure the effectiveness of treatment (Knuuti et al., 2020). Currently, the standard of treatment for stable exertional angina includes a number of metabolic drugs that provide a cardiocytoprotective effect (Knuuti et al., 2020). The direction of cytoprotective pharmacotherapy is traditionally considered to be of secondary importance in the treatment of hypoxic conditions associated with tissue ischemia, including angina pectoris (Berezhnova, Dyadina, & Kulintsova, 2020; Knuuti et al., 2020; Semeleva, Blinova, Zaborovsky, Gromova, & Shukurov, 2020). The ambiguous efficacy of

doi.org/10.31838/hiv22.02.15 Received: 09.07.21, Revised: 12.08.21, Accepted: 15.09.21

cytoprotectors discovered by a number of authors may indicate the need for a personalized approach to prescribing this group of drugs (Novikov, Levchenkova, & Ivantsova, 2020; OlesyaV Romaschenko et al., 2021; Romashchenko, 2018). Currently, a whole area of personalized pharmacotherapy is actively developing (Petrov, Shishimorov, Magnitskaya, & Tokachev, 2016).

The use of an antioxidant drug in the complex pharmacotherapy of CHD can increase the effectiveness of treatment (O. Romaschenko et al., 2020), since myocardial ischemia is associated with coronary atherosclerosis and activation of lipid peroxidation (Heusch, 2019). The antioxidant property of the drug pathogenetically can lead to cytoprotective and energy-saving effects (Kukes, Gorbach, Romashchenko, & Rumbest, 2014), which would be important in case of myocardial ischemia. In this regard, it is of particular interest to study the effect of the antioxidant drug etoxidol on cell viability and the energy state of cell mitochondria in patients with CHD.

The experimental model for studying the viability of cells was the blood leukocytes of patients, since they can reflect the internal state of the human body and are readily available material for research. These immune cells are considered as a kind of "mirror of homeostasis", which can be used to determine the nature of the process underlying the disease, its severity, prognosis and effectiveness of therapy (Uzenbaeva, Kizhina, Ilyukha, Belkin, & Khizhkin, 2019). Moreover, W. Jin, G. Deng-Feng, W. Hao et al, based on a number of their own studies, argue that the nature of mitochondrial damage in cardiomyocytes and peripheral blood leukocytes is identical, leukocytes reflect changes in cardiomyocytes, as in a mirror (Wei et al., 2014).

PURPOSE

The aim of this study was to determine the cytoprotective properties of the antioxidant drug ethoxidol on the basis of an in vitro study of blood cells viability in patients with CHD.

MATERIALS AND METHODS

We examined 30 patients with coronary heart disease: stable angina pectoris of I-III functional classes (acute coronary syndrome was excluded from the study), who were admitted to the Department of Cardiology No. 1 of the Belgorod Regional Clinical Hospital of St. Joasaph from January to June 2019. The study group included 20 women and 10 men aged from 49 to 81 years, the average age of patients was 66.0 ± 2.0 years old.

Blood sampling was performed in the morning on an empty stomach in a vacuum tube with ethylenediamine tetraacetic acid (EDTA). A prerequisite for the selection of patients for the study was the absence of X-rays for at least 21 days before blood sampling due to the well-known destructive effect of X-rays on human leukocytes and the ability of white blood cells to completely renew the composition within 21 days with an average life expectancy of leukocytes of 7-9 days (Brubaker, Le Roy, & Mengel, 1977).

To determine the viability of blood cells, leukocytes (0.5 ml) were collected manually with a micropipette under aseptic conditions, mixed with 2 ml of RPMI-1640 culture medium with glutamine (PanEko, Russia), then placed into the wells of a 24-well plate, 20 µl of leukocyte suspension in each well. The culture medium and the drug were added in an amount necessary to create a therapeutic concentration of ethoxidol in the well of 0.68 µg / ml. We were guided by the official instructions for the medical use of ethoxidol. Then the samples were incubated for 3 hours (time sufficient for the drug to interact with cells) in an incubator with 5% CO_2 content at a temperature of 37°C (conditions of the human internal environment). After 3 hours of incubation, 500 µl of the supernatant was taken from each well and fluorescent dyes were added to the remaining 500 µl at a final concentration of 1nM/µl for MitoTracker ™ Red CMXRos (Invitrogen, USA), which allows to evaluate the mitochondrial membrane potential and Calcein AM (Invitrogen , USA), which stains only viable cells and at a final concentration of 2nM/µL for Ethidium bromide (Sigma-Aldrich, USA), which stains only dead cells (Larionov, Malov, Mandrik, Maslov, & Orishich, 2003). The samples were again placed in a thermostat under the same conditions for another 30 minutes (time sufficient for staining the cells). When developing the scheme of the experiment, we were guided by the tutorial of (Mitroshina, Mishchenko, & Vedunova, 2015).

The results were evaluated by fluorescence microscopy using an Eclipse Ti-U inverted microscope (Nikon, Japan). The data were processed using the specialized software EZ-C1 FreeViewer Ver3.90 (Nikon), the fluorescence intensity in 100 cells in 10 fields of view for each well was determined, expressed in relative units.

The number of living and dead cells was counted, the cells viability index was calculated using the formula:

$$VI_{cells} = (Z_{living\ cells} - Z_{dead\ cells}) / (Z_{dead\ cells}) * 100, \quad (1)$$

where VI_{cells} is the cells viability index (in %),

$Z_{living\ cells}$ - the number of living cells in 10 fields of view

$Z_{dead\ cells}$ - the number of dead cells in 10 fields of view

By the nature of the change in the cells viability index under the influence of the drug administered in vitro, the presence of the cytoprotective properties of ethoxidol was judged

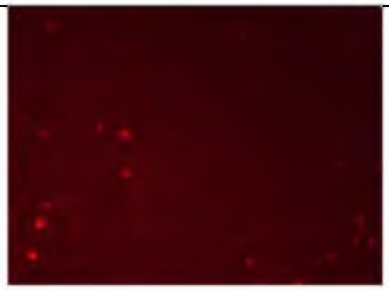
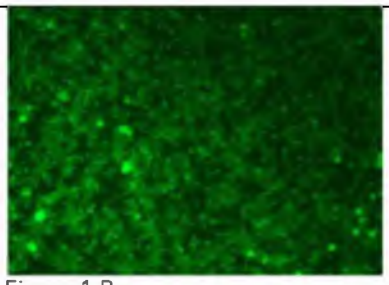
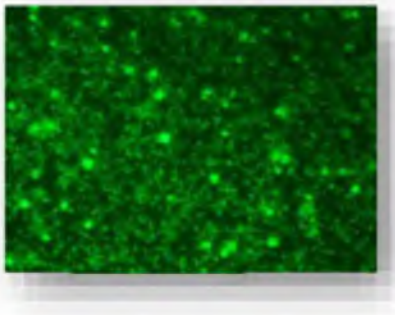
according to the method we developed (O. V. Romaschenko et al., 2022).

A total of 12,000 cells were analyzed. The materials were processed statistically with the calculation of the arithmetic mean, error of the mean, assessment of the significance of differences by Student's t-test. Correlation analysis was also performed.

The study was carried out on the basis of the laboratory of cell technologies of the Research Institute of Pharmacology of Living Systems, Belgorod State University.

RESULTS AND DISCUSSION

In the control wells (without adding the drug), when analyzing the fluorescence of the mitochondria of blood leukocytes stained by Mito Tracker Red, clearly glowing cells, round and shaped, were found, which indicates normally functioning mitochondria (Fig. 1, A.). The average mitochondria fluorescence value in the group was 120.20 ± 4.18 rel.units. Simultaneous staining of samples by Calcein AM showed that all luminous cells with functioning mitochondria are alive (Fig. 1, B.). The number of dead cells in relation to the total number of analyzed cells was 30%. (Fig. 1, C.). The index of viability of leukocytes in patients with CHD, on average for the group (without the introduction of ethoxidol), was 41%.

	without ethoxidol	with ethoxidol (0.68 µg / ml)
Luminescence of blood leukocytes mitochondria (dye - Mito Tracker Red). Fluorescence microscopy, magnification X200.	 Figure 1.A.	 Figure 2.A.
Luminescence of membrane structures of living white blood cells (dye - Calcein AM). Fluorescence microscopy, magnification X200.	 Figure 1.B.	 Figure 2.B.
Luminescence of dead leukocytes (dye - Ethidium bromide). Fluorescence microscopy, magnification X200	 Figure 1.C.	 Figure 2.C.

Fluorescence microscopy of blood leukocytes of patients with CHD in the wells, which were supplemented with etoxidol at a therapeutic concentration and the dye Mito Tracker Red, revealed clearly glowing cells, round and shaped, which indicates normally functioning mitochondria (Fig. 2, A.) The value of mitochondrial fluorescence the average for the group was 127.64 ± 5.41 relative units, which is higher in comparison with the control (120.20 ± 4.18 relative units), but without reaching the level of significance of differences ($p > 0.05$).

The staining of the same Calcein AM samples showed that all luminous cells with functioning

mitochondria are alive (Fig. 2, B.). The number of dead cells in relation to the total number of analyzed cells was 28% (Fig. 2.C) The leukocyte viability index in patients with CHD, on average in the group, after the introduction of ethoxidol increased by 21% (from 41% to 62%, p <0.001), which indicates the presence of cytoprotective properties in this drug (O. V. Romaschenko et al., 2022).

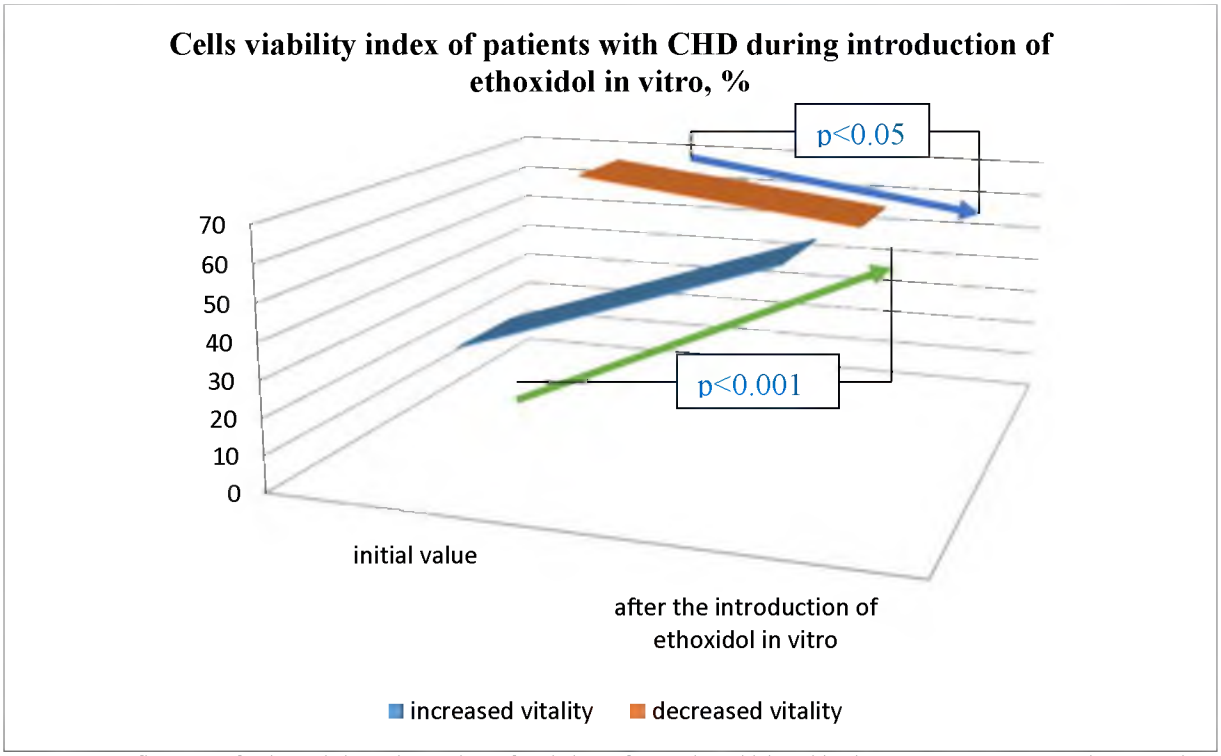


Figure 3. Influence of ethoxidol on the index of viability of peripheral blood leukocytes in patients with CHD (therapeutic concentration 0.68 μg / ml, 3 hours of incubation, in vitro).

A more detailed analysis of the dynamics of the VI $_{cells}$ index showed two variants of changes in cell viability: in 80% of patients, VI $_{cells}$ was increased, on average, by 28% (from 36% to 64%, p <0.001) and in 20% of patients, VI $_{cells}$ was decreased, on average, by 10% (from 68% to 58%, p <0.05), at the same time, the groups significantly differed in the initial level of cell viability (p<0.01) (Fig.3.).

The value of mitochondrial fluorescence changed in both cases upward, but without achieving the reliability of differences (Table 1.). At the same time, the groups significantly differed in the initial level of mitochondrial fluorescence: in the group with an increase in cell vitality under the influence of ethoxidol, administered in vitro, an initially lower value of mitochondrial fluorescence intensity was noted (Table 1.)

Table 1.Mitochondrial fluorescence intensity in patients with CHD during in vitro introduction of ethoxidol

Indicator	Decreased vitality		Increased vitality	
	Initial value	After the introduction of ethoxidol	Initial value	After the introduction of ethoxidol
Mitochondrial fluorescence intensity, relative units	144.14±10.81**	149.84±16.55*	114.17±3.63**	123.33±5.06*

Note. **Significant differences between the comparison groups at baseline (p<0.01); *Significant differences between the comparison groups after in vitro introduction of ethoxidol (p <0.05).

Correlation analysis showed no dependence of cell viability on the fluorescence intensity of their mitochondria in the case of positive dynamics from the introduction of ethoxidol: the correlation coefficient (CC) was 0.12 (p> 0.05) both in the initial state and after in vitro introduction of ethoxidol. In the case of negative dynamics from the introduction of ethoxidol, a highly significant negative correlation relationship was found between the indicators of cell viability and the intensity of fluorescence of their mitochondria both in the initial state (the CC was -0.65 (p <0.01) and after in vitro introduction of ethoxidol (the CC was -0.97 (p <0.01)), and, as can be seen from the values of the correlation coefficients, the strength of the relationship increased from moderate to

strong after the introduction of ethoxidol into the wells in vitro.

The pathogenetic substantiation of the validity of our results and conclusions are the following theoretical provisions ... The main cause of myocardial ischemia is atherosclerosis of the coronary vessels (Rocha, Rocha, Succi, & Brito Junior, 2020). At the initial stages of atherosclerosis, atherogenic hyperlipoproteinemia is observed (Ference, Graham, Tokgozoglu, & Catapano, 2018). In the presence of vascular endothelial dysfunction (increased endothelial permeability due to activation of lipid peroxidation), atherogenic low density lipoproteins enter the vascular wall. An excess of lipids in the cell promotes further activation of lipid peroxidation, as a result of which cholesterol becomes foreign to the cell and immune mechanisms of atherosclerosis progression are triggered (Wu, Li, Hou, & Chu, 2017).

Lipid peroxidation is a branched chain reaction involving reactive oxygen species (free radicals). A free radical is a highly reactive molecular particle that has an unpaired electron in its outer orbital. Lipid peroxidation of membranes leads to disruption of cellular homeostasis: a decrease in the synthesis of ATP, DNA, RNA, activation of proteolytic enzymes, cytolysis, and, ultimately, cell death (Gaschler & Stockwell, 2017).

An antioxidant, due to the presence of unpaired electrons in its molecule, is able to capture electrons of reactive oxygen species and neutralize them, thus preventing damage to cell membranes and other structures - mitochondria, DNA molecules, RNA, maintaining normal ATP production and cell viability (Shivakumar & Yogendra Kumar, 2018).

According to the official instructions for medical use and literature data, ethoxidol has a number of pleiotropic effects, including hypolipidemic, antiplatelet, antiischemic, membrane stabilizing, endothelioprotective, etc. (Kukes et al., 2014; O. Romaschenko et al., 2020). Many research works are devoted to the pharmacological correction of endothelial dysfunction; endothelioprotection is an important pharmacodynamic target [(M. Korokin et al., 2019; M. Korokin et al., 2020; M. V. Korokin et al., 2015; M. V. Korokin et al., 2019; Stepchenko et al., 2020). Apparently, all these pleiotropic effects are a consequence of the direct antioxidant action of ethoxidol.

The ability of ethoxidol to influence on cell viability in different ways, depending on the initial level of their viability and the intensity of mitochondrial fluorescence, discovered by us, is new, requires further study and indicates the need to develop personalized approaches to prescribing this drug to patients with coronary heart disease.

CONCLUSION

Thus, according to the results of our in vitro study, the antioxidant drug ethoxidol reliably possesses cytoprotective properties when used in patients with coronary heart disease; however, there is some variability in the change in the viability of cells in patients with CHD for in vitro administration of ethoxidol in the form of an increase (by 80% cases) or a decrease (in 20% of cases) of their viability, which indicates the need for a personalized approach to prescribing this drug to patients with CHD.

1. With the introduction of ethoxidol into a sample with a leukocyte suspension, a significant increase in the viability index (VI_{cells}) by 21% (from 41% to 62%, $p < 0.001$) was observed, which indicates the presence of cytoprotective properties in this drug.
2. A more detailed analysis of the dynamics of the VI_{cells} index showed two variants of changes in cell viability: in 80% of patients VI_{cells} was increased, on average, by 28% (from 36% to 64%, $p < 0.001$) and in 20% of patients VI_{cells} was decreased, on average, by 10% (from 68% to 58%, $p < 0.05$). The groups significantly differed in the initial level of cell viability (36% versus 68%, $p < 0.01$), and in the initial level of mitochondrial fluorescence intensity: in the group of increasing cell viability under the influence of ethoxidol, the initial value of mitochondrial fluorescence was significantly lower (114.17 ± 3.63) in comparison with a group of decreased viability (144.14 ± 10.81, $p<0.01$).
3. The value of mitochondrial fluorescence after the introduction of ethoxidol, changed in both cases upward, but without achieving the reliability of differences. No clear relationship was found between the indicators of cell viability and the value of fluorescence of their mitochondria in the case of positive dynamics from ethoxidol (the correlation coefficient (CC) was 0.12; $p > 0.05$) and, at the same time, in the case of negative dynamics from the introduction of ethoxidol, a highly significant negative correlation relationship was found between the indicators of cell viability and the fluorescence intensity of their mitochondria as in the initial state (the CC was -0.65 ($p < 0.01$), and after in vitro introduction of ethoxidol (the CC was -0.97 ($p < 0.01$)), moreover, the strength of the relationship increased from moderate to strong after the introduction of ethoxidol into the wells in vitro.

REFERENCES

Berezhnova, T. A., Dyadina, K. S., & Kulintsova, Y. V. (2020). Immune-metabolic therapy of purulent inflammatory diseases. *6*(4), 1-6. doi:https://doi.org/10.3897/rrpharmacology.6.55628

Bontsevich, R. A., Filinichenko, T. S., Gavrilova, A. A., Goncharova, N. Y., & Myronenko, O. V. (2018). Assessment of physicians' and senior medical students' knowledge in treatment of patients with community-acquired pneumonia: Current results of the KNOCAP

project. *4*(3), 27-36. doi:https://doi.org/10.3897/rrpharmacology.4.29454
Bontsevich, R. A., Gavrilova, A. A., Adonina, A. V., Vovk, Y. R., Goncharova, N. Y., Batisheva, G. A., . . . Barysheva, V. O. (2020). Rational antimicrobial chemotherapy: assessment of the level of basic knowledge of general practitioners. Final results of the KANT project. *Research Results in Pharmacology, 6*(1), 93-99. doi:https://doi.org/10.3897/rrpharmacology.6.54855
Brubaker, L. H., Le Roy, J. E., & Mengel, C. E. (1977). Neutrophil life span in paroxysmal nocturnal hemoglobinuria. *Blood, 50*(4), 657-662. doi:https://doi.org/10.1182/blood.V50.4.657.657
Ference, B. A., Graham, I., Tokgozoglu, L., & Catapano, A. L. (2018). Impact of lipids on cardiovascular health: JACC health promotion series. *Journal of the American College of Cardiology, 72*(10), 1141-1156. doi:https://doi.org/10.1016/j.jacc.2018.06.046
Gaschler, M. M., & Stockwell, B. R. (2017). Lipid peroxidation in cell death. *Biochemical and biophysical research communications, 482*(3), 419-425. doi:https://doi.org/10.1016/j.bbrc.2016.10.086
Gavrilova, A. A., Bontsevich, R. A., Vovk, Y. R., & Balabanova, A. A. (2020). Modern approaches to pharmacotherapy of community-acquired pneumonia. *6*(4), 77-84. doi:https://doi.org/10.3897/rrpharmacology.6.52318
Gruzdeva, A. A., Khokhlov, A. L., & Ilyin, M. V. (2020). Risk management strategy for preventing the reduced treatment effectiveness from the position of drug interactions and polypharmacy in patients with coronary heart disease. *6*(4), 85-92. doi:https://doi.org/10.3897/rrpharmacology.6.60164
Heusch, G. (2019). Myocardial ischemia: lack of coronary blood flow, myocardial oxygen supply-demand imbalance, or what? *American Journal of Physiology-Heart and Circulatory Physiology, 316*(6), H1439-H1446. doi:https://doi.org/10.1152/ajpheart.00139.2019
Knuuti, J., Wijns, W., Saraste, A., Capodanno, D., Barbato, E., Funck-Brentano, C., . . . Cuisset, T. (2020). 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes: the Task Force for the diagnosis and management of chronic coronary syndromes of the European Society of Cardiology (ESC). *European heart journal, 41*(3), 407-477. doi:https://doi.org/10.1093/eurheartj/ehz425
Kontsevaya, A., Bobrova, N., Barbarash, O., Duplyakov, D., Efanov, A., Galyavich, A., . . . Mirolyubova, O. (2017). The management of acute myocardial infarction in the Russian Federation: protocol for a study of patient pathways. *Wellcome open research, 2*(89). doi:https://doi.org/10.12688/wellcomeopenres.12478.1
Korokin, M., Gudyrev, O., Gureev, V., Korokina, L., Peresypkina, A., Pokrovskaia, T., . . . Pokrovskii, M. (2019). Studies to elucidate the effects of furostanol glycosides from dioscorea deltoidea cell culture in a rat model of endothelial dysfunction. *Molecules, 25*(1), 169. doi:https://doi.org/10.3390/molecules25010169
Korokin, M., Gureev, V., Gudyrev, O., Golubev, I., Korokina, L., Peresypkina, A., . . . Zatolokina, M. (2020). Erythropoietin mimetic peptide (pHBSP) corrects endothelial dysfunction in a rat model of preeclampsia. *International journal of molecular sciences, 21*(18), 6759. doi:https://doi.org/10.3390/ijms21186759
Korokin, M. V., Pokrovskii, M. V., Gudyrev, O. S., Korokina, L. V., Pokrovskaia, T. G., Lazarev, A. I., . . . Gureev, V. V. (2015). Pharmacological correction of endothelial dysfunction in rats using e-NOS cofactors. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6*(5), 1548-1552.
Korokin, M. V., Soldatov, V. O., Tietze, A. A., Golubev, I. V., Belykh, A. E., Kubekina, M. V., . . . Tat'Yana, G. P. (2019). 11-amino acid peptide imitating the structure of erythropoietin α-helix b improves endothelial function, but stimulates thrombosis in rats. ***Фармация и фармакология,*** *7*(6 (eng)), 312-320. doi:https://doi.org/10.19163/2307-9266-2019-7-6-312-320
Kukes, V. G., Gorbach, T. V., Romashchenko, O. V., & Rumbest, V. V. (2014). Energy-saving activity of the antioxidant ethoxidol in simulated myocardial ischemia. *Drugs and rational pharmacotherapy, 1*, 16-20.
Larionov, P. M., Malov, A. N., Mandrik, M. M., Maslov, N. A., & Orishich, A. M. (2003). Changes in the spectrum of laser-induced fluorescence of myocardial tissue as its viability decreases. *Journal of Applied Spectroscopy, 70*(1), 38-42. doi:https://doi.org/10.1023/A:1023212206592
Mitroshina, E. V., Mishchenko, T. A., & Vedunova, M. V. (2015). Determination of the viability of cell cultures. Study guide. *Nizhny Novgorod.-21p.*
Novikov, V. E., Levchenkova, O. S., & Ivantsova, E. N. (2020). Possibilities of antihypoxant use for mitochondrial dysfunctions. *Bulletin of the Smolensk State Medical Academy, 19*(1), 41-55.
Petrov, V. I., Shishimorov, I. N., Magnitskaya, O. V., & Tokachev, B. E. (2016). Personalized medicine: the evolution of methodology and problems of practical implementation. *Bulletin of Volgograd State Medical University, 1*(57), 3-11.
Rocha, L. O., Rocha, E., Succi, G. d. M., & Brito Junior, R. B. d. (2020). Association between Periodontitis, Genetic Polymorphisms and Presence of Coronary Artery Disease in Southern Brazil. *Arquivos brasileiros de cardiologia, 114*, 268-272.
Romaschenko, O., Gorbach, T., Alferov, P., Grischenko, N., Pokrovskaya, T., & Rumbesht, V. (2020). Endothelioprotective effect of a combination of statin with antioxidant in the treatment of patients with coronary heart disease. *7*(10), 571-575.
Romaschenko, O., Pokrovsky, M., Nadezhdin, S., Rumbesht, V., Zhernakova, N., Alferov, P., . . . Kazakova, E. E. (2021). Personalized Approaches To The Use Of Meldonium As A Cytoprotector In Patients With Coronary Heart Disease. *NVEO-NATURAL VOLATILES & ESSENTIAL OILS Journal| NVEO*, 3675-3685.
Romaschenko, O. V., Pokrovsky, M. V., Nadezhdin, S. V., Rumbesht, V. V., Zhernakova, N. I., Alferov, P. K., . . . Kukes, V. G. (2022). Personalized Approaches to the Use of the Antioxidant Ethoxidol in Patients with Coronary Heart Disease. *Journal of Nanostructures, 12*(2), 343-352.
Romashchenko, O. V. (2018). Influence of cytoflavin on the DNA of blood leukocytes of patients with ischemic heart disease depending on the polymorphism of the endothelial nitric oxide synthase gene. *Eksperimental'naya i Klinicheskaya Farmakologiya, 81*(6), 14-19.
Semeleva, E. V., Blinova, E. V., Zaborovsky, A. V., Gromova, I. A., & Shukurov, A. S. (2020). Metal-containing taurine compounds protect rat's brain in reperfusion-induced injury. doi:https://doi.org/10.3897/rrpharmacology.6.59857
Shivakumar, A., & Yogendra Kumar, M. S. (2018). Critical review on the analytical mechanistic steps in the

evaluation of antioxidant activity. *Critical reviews in analytical chemistry, 48*(3), 214-236. doi:https://doi.org/10.1080/10408347.2017.1400423
SK, Z., SB, F., AV, V., II, S., NN, S., DA, K., . . . YG, V. (2020). Medication adherence in patients with stable coronary artery disease in primary care. Research Results in Pharmacology., *6*(2), 97-103. doi:https://doi.org/10.3897/rrpharmacology.6.54130
Stepchenko, A. A., Pokrovskaia, T. G., Gudyrev, O. S., Soldatov, V. O., Pokrovskii, M. V., Batishcheva, G. A., . . . Gureev, V. V. (2020). Eleven-amino acid peptides that mimic the erythropoietin α-helix B increases cell survival in endotheliocyte culture. *Archivos Venezolanos de Farmacologia y Terapeutica, 39*(5), 533-540.
Uzenbaeva, L. B., Kizhina, A. G., Ilyukha, V. A., Belkin, V. V., & Khizhkin, E. A. (2019). Morphology and composition of peripheral blood cells during hibernation in bats (chiroptera, vespertilionidae) of northwestern Russia. *Biology Bulletin, 46*(4), 398-406. doi:https://doi.org/10.1134/S1062359019030130
Wei, J., Gao, D.-F., Wang, H., Yan, R., Liu, Z.-Q., Yuan, Z.-Y., . . . Chen, M.-X. (2014). Impairment of myocardial mitochondria in viral myocardial disease and its reflective window in peripheral cells. *PLoS One, 9*(12), e116239.
doi:https://doi.org/10.1371/journal.pone.0116239
Wu, M.-Y., Li, C.-J., Hou, M.-F., & Chu, P.-Y. (2017). New insights into the role of inflammation in the pathogenesis of atherosclerosis. *International journal of molecular sciences, 18*(10), 2034. doi:https://doi.org/10.3390/ijms18102034
Zyryanov, S. K., Fitilev, S. B., Vozzhaev, A. V., Shkrebniova, I. I., & Klyuev, D. A. (2020). Critical aspects of the management of stable coronary artery disease in primary care practice or how to increase the efficacy of evidence-based pharmacological therapy? , *6*(3), 15-20. doi:https://doi.org/10.3897/rrpharmacology.6.53615