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ENDOTHELIOPROTECTIVE EFFECT OF A COMBINATION OF STATIN WITH ANTIOXIDANT IN THE TREATMENT OF PATIENTS WITH CORONARY HEART DISEASE

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ABSTRACT

Introduction: The widespread prevalence and mortality from coronary heart disease (CHD) determine the urgency of the problem of finding rational drug combinations for more effective treatment of this pathology. Endothelium of the vessels is the target organ for pharmacological correction of cardiovascular pathology. We suggested that the combination of statin with an antioxidant may be rational in terms of endothelial dysfunction correction in the treatment of patients with coronary artery disease, which this work is devoted to. **Research tasks:** The aim of this research was to study the rationality of the combination of statin (atorvastatin) and antioxidant (ethoxidol) in relation to endothelioprotection in the treatment of patients with CHD. **Material and Methods:** We conducted a prospective, open, randomized controlled comparative study of 60 patients with CHD: stable angina pectoris, which were divided into two groups: 1st (control, n = 30) - patients who received basic therapy for stable angina pectoris with an average dose of atorvastatin 20mg per day and 2nd (study group, n = 30) - patients receiving additional ethoxidol at a dose of 300 mg per day in tablets for 50-54 days while reducing the dosage of atorvastatin to 10 mg per day. In addition, 20 practically healthy individuals were examined. Endothelial function was evaluated based on a study of three indicators in the blood - the Willibrandt factor (FVB) by photoelectrocolorimetry, as well as endothelin-1 and endothelial nitric oxide synthase (e-NOS) by enzyme-linked immunosorbent assay. The endothelial function index was calculated as the ratio of the vasodilation marker and vasoconstriction marker: e-NOS / endothelin-1. Materials were processed statistically. **Results:** the activity of FVB during the treatment significantly decreased - in the 1st group by 42% ($p<0.001$) and in the 2nd group by 50% ($p<0.001$); no differences between the groups were observed. The level of endothelin-1 in the blood also significantly decreased in the dynamics of treatment of patients with CHD: in the 1st group from 29.32±3.22 pkg/ml to 17.54±1.88 pkg/ml ($p<0.001$), and in the 2nd group - from 26.71±3.69 pkg/ml to 16.61±2.14 pkg/ml ($p<0.05$), while in the 1st and 2nd groups the level of endothelin-1 was not achieved healthy individuals (8.47±1.18 pcg / ml). The level of eNOS significantly increased during the treatment in both groups of patients: in the 1st group from 82.69±6.52 pkg/ml to 156.40±14.18 pkg/ml ($p<0.001$) and in the 2nd group - from 85.17±5.37 pkg/ml to 148.16±9.47 pkg/ml ($p<0.001$), without reaching the level of eNOS of practically healthy individuals (222.97±11.38 pkg/ml). The endothelial function index increased during the treatment in both groups: in the 1st - from 2.82 to 8.92 ($p<0.001$) and in the 2nd - from 3.19 to 8.92 ($p<0.001$), also without reaching the level of this indicator for healthy individuals (26.32). **Conclusion:** It has been obtained the evidence of the rationality of the combination of statin with an antioxidant in achieving the endothelioprotective effect of treatment of patients with CHD. Reducing the dosage of atorvastatin while taking the antioxidant drug ethoxidol allows you to achieve a comparable endothelioprotective effect.

KEYWORDS: endothelioprotection, coronary heart disease, treatment, patients, statins (atorvastatin), antioxidants (ethoxidol).

1. INTRODUCTION

The widespread prevalence and mortality from coronary heart disease (CHD) determine the urgency of the problem of finding rational drug combinations for more effective treatment of this pathology [1]. Among the basic agents that improve the prognosis for chronic coronary artery disease, statins appear as the main group of lipid-lowering drugs with proven effectiveness [2,3]. A decrease in blood cholesterol is accompanied by a significant population-related decrease in overall mortality and the risk of all cardiovascular complications [4]. Perhaps this population effect from the use of statins is associated not only with their hypolipidemic activity, but also with a number of proven pleiotropic effects, among which endothelium protection is listed [5,6]. The pleiotropic effects of drugs often prove to be quite useful [7,8]. Endothelium of the vessels is the target organ for pharmacological correction of cardiovascular pathology both in the clinic and in the experiment [9-16]. Earlier in the experiment, we discovered an endothelioprotective effect in a number of antioxidants, including ethoxidol, the subject of our study [17,18]. The rationality of the combination of statin with an antioxidant in endothelial dysfunction was also shown by us in an animal experiment [19]. We suggested that the combination of statin with an antioxidant may be rational in terms of endothelial dysfunction correction in the treatment of patients with coronary artery disease,

which this work is devoted to. The purpose of this study was to study the rationality of the combination of statin (atorvastatin) and antioxidant (ethoxidol) in relation to endothelioprotection in the treatment of patients with CHD.

2. MATERIALS AND METHODS

To achieve the goal of the study, we conducted a prospective, open, randomized, controlled, comparative study of 60 patients with CHD: stable angina pectoris, I-III functional classes. Patients were divided into two equivalent groups by randomization - 30 people in each group:

1st (control) – patients, who received basic therapy for stable angina pectoris according to the recommendations of Europe Society of Cardiology (2013) [2]. In order to improve the prognosis of CHD in patients there were prescribed antiplatelet drugs (aspirin at a dose of 75 mg/day), lipid-lowering drugs (atorvastatin at a dose of 20 mg/day), beta-blockers, ACE inhibitors. In order to stop the symptoms of angina pectoris and improve the quality of life there were prescribed antianginal drugs, calcium antagonists and nitrates;

2nd (study group) – patients, who received additional ethoxidol at a dose of 100 mg 3 times a day in tablets under the tongue for 10-14 days of inpatient treatment and 40 days outpatiently during basic therapy. The basic therapy in this observation group changed on during outpatient treatment - the dosage of atorvastatin was reduced to ½ to 10 mg/day (in a hospital these patients received atorvastatin at a dose of 20 mg/day). The groups were comparable by sex, age, and severity of the underlying pathology and basic drug therapy. In addition, 20 practically healthy individuals were examined, comparable in gender and age with the studied patients. The duration of observation of patients was up to 2 months (inpatient plus outpatient observation period) with an assessment of the condition of patients in the initial state and at the end of the outpatient observation phase.

In order to assess correctly the endothelioprotective effect of pharmacotherapy in the comparison groups, we analyzed three main indicators of endothelial function - Willibrandt factor (FVB), endothelin-1, endothelial nitric oxide synthase (e-NOS). A detailed description of these indicators, their research methods and standards are given in table 1.

Table 1.: The studied indicators of endothelial function

Indicator	Biological effect	Research method	Normal (level in healthy individuals)
Willibrandt factor (FVB)	- a recognized marker of endothelial dysfunction, it is synthesized by endothelial cells and megakaryocytes, it is necessary for normal platelet adhesion and has the ability to lengthen the half-life of the VIII coagulation factor. An increase of FVB in plasma is observed with lesions of the vascular wall due to the increased release of FVB from the endothelium into the blood.	Determination of PVB activity in blood plasma on a photoelectrocalorimeter (in %)	50-150%
Endothelin-1	- a vasoconstrictive peptide that plays a key role in blood vessel homeostasis. Endothelin is the most powerful known vasoconstrictor agent. Endothelin - 1 is the most active isomer with which many pathological conditions of the circulatory system are associated.	linked immunosorbent assay	8.47 ± 1.18 pkg/ml
Endothelial nitric oxide synthase (e-NOS)	Nitric oxide endothelial synthase is an enzyme that catalyzes the formation of nitric oxide in endothelial cells. Nitric oxide is a known endothelial vasodilating factor.	linked immunosorbent assay	222.97 ± 11.38 pkg/ml

In order to objectify the assessment of endothelial function, the endothelial function index was calculated as the ratio of the vasodilation marker and vasoconstriction marker: eNOS / endothelin-1.

Materials were processed statistically. The results were considered statistically significant at $p < 0.05$. Quantitative indicators were evaluated for compliance with the normal distribution using the Kolmogorov-Smirnov test. For indicators with a distribution close to normal, the arithmetic mean value, standard deviation, and mean error were calculated as the ratio of the standard deviation to the square root of the number of analyzed

values. These results are presented in the table in the form of "mean ± error of the mean." Differences between two dependent groups of observations (before and after treatment) by a quantitative characteristic having a distribution close to normal were evaluated using Student's t-test for dependent samples. When carrying out the calculations, the program “Microsoft Excel 2016” was used.

3. RESULTS AND DISCUSSION

The results of the study are presented in table 2 and in figure 1.

Table 2.: Endothelial function indicators in patients with coronary heart disease in the dynamics of treatment

Indicator	1st group (atorvastatin 20mg/day)		2nd group (atorvastatin 10mg/day + ethoxidol)		healthy individuals
	initial state	after treatment	initial state	after treatment	
FVB, %	142.90±7.06**	100.10±3.92**	151.67±6.18**	101.81±2.63**	-
Endothelin-1, pkg/ml	29.32±3.22**!	17.54±1.88**!	26.71±3.69*!	16.61±2.14*!	8.47 ± 1.18
e-NOS, pkg/ml	82.69±6.52**!	156.40±14.18**!	85.17±5.37**!	148.16±9.47**!	222.97 ± 11.38

Note. * p <0.05; ** p <0.001 - significance of differences before and after treatment within the group; !p <0.001 - significance of differences with a group of healthy individuals.

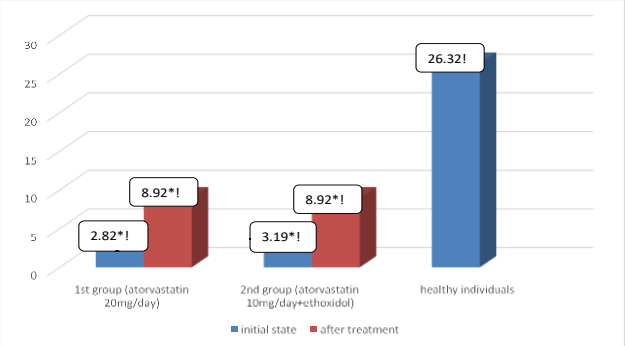

Figure 1. The index of endothelial function in the observation groups in the dynamics of treatment in comparison with practically healthy people. Note. * p <0.001 - significance of differences before and after treatment within the group; ! p<0.001 - significance of differences with a group of healthy individuals.

As can be seen in table 2, the activity of the Willibrandt factor as an indicator of endothelial dysfunction in the treatment dynamics significantly decreased - in the 1st group by 42% (p <0.001) and in the 2nd - by 50% (p <0.001); no differences between the groups were observed. The level of vasoconstrictor factor endothelin-1 in the blood also significantly decreased during the treatment of patients with CHD: in the 1st group from 29.32±3.22 pkg/ml to 17.54±1.88 pkg/ ml (p <0.001), and in the 2nd group - from 26.71±3.69 pkg/ml to 16.61±2.14 pkg/ml (p <0.05), while both in the 1st and 2nd groups the level of endothelin-1 was not achieved the level of healthy individuals (8.47±1.18 pkg/ml), which is completely explained by the potential impossibility to restore the function of the endothelium of damaged vessels fully in coronary heart disease. At the same time, the level of endothelial nitric oxide synthase, which catalyzes the formation of endothelial vasodilation factor, significantly increased in the dynamics of treatment of both groups of patients: in the 1st group, from 82.69±6.52 pkg/ml to 156.40±14.18 pkg/ml (p <0.001) and in the 2nd group - from 85.17±5.37 pkg/ml to 148.16±9.47 pkg/ml (p<0.001), while not reaching the level of eNOS of practically healthy individuals (222.97±11.38 pkg/ml). The endothelial function index was increased during the treatment in both groups: in the 1st - from 2.82 to 8.92 (p<0.001) and in the 2nd - from 3.19 to 8.92 (p <0.001), also without reaching the level of this indicator for healthy individuals (26.32) (Figure 1.). An analysis of the data presented allows us to draw the following conclusion: in both observation groups, the endothelioprotective effect of pharmacotherapy with correction of endothelial function is noted, but without reaching the level of practically healthy individuals. Groups are comparable in terms of endothelial protection. Reducing the dosage of statin while taking the antioxidant drug ethoxidol allows you to achieve a comparable endothelioprotective effect. The pathogenetic rationale for the rationality of such a combination of drugs is the following theoretical provisions ...

The main cause of myocardial ischemia is coronary atherosclerosis [20]. At the initial stages of atherosclerosis, atherogenic hyperlipoproteinemia is observed [21]. In the presence of vascular endothelial dysfunction (increased endothelial permeability due to activation of lipid peroxidation) atherogenic LDL enters the vascular wall. Excess lipids in the cell contributes to the further activation of lipid peroxidation, as a result of which cholesterol becomes foreign to the cell and the immune mechanisms of atherosclerosis progression are

triggered [22]. Lipid peroxidation is a branched chain reaction involving active oxygen species (free radicals). A free radical is a molecular particle that has an unpaired electron on the outer orbital and is highly reactive. The lipid peroxidation of membranes leads to disruption of cell homeostasis: a decrease in the synthesis of ATP, DNA, RNA, activation of proteolytic enzymes, cytolysis and, ultimately, cell death [23].

The antioxidant, due to the presence of unpaired electrons in its molecule, is able to trap electrons of reactive oxygen species and neutralize them, thus preventing damage to cell membranes and other structures - mitochondria, DNA molecules, RNA, while maintaining normal ATP production and cell viability [24]. Thus, a pharmacological strategy aimed at combining a lipid-lowering drug and an antioxidant, which can provide the most effective correction of the atherosclerotic process at the level of the endothelial vascular wall, is pathogenetically justified.

CONCLUSION

In both observation groups of patients with CHD – treated by basic therapy with atorvastatin 20 mg/day and treated by complex therapy with atorvastatin 10 mg/day plus antioxidant etoxidol, the endothelioprotective effect of pharmacotherapy with correction of endothelial function is noted, but without reaching the level of practically healthy individuals. Groups are comparable in terms of endothelial protection. Reducing the dosage of statin while taking the antioxidant drug ethoxidol allows you to achieve a comparable endothelioprotective effect.

MAIN FINDINGS

1. In both observation groups of patients with CHD – treated by basic therapy with atorvastatin 20 mg/day and treated by complex therapy with atorvastatin 10 mg/day plus antioxidant etoxidol, the endothelioprotective effect of pharmacotherapy with correction of endothelial function is noted, but without reaching the level of practically healthy individuals.
2. Reducing the dosage of atorvastatin to the minimum (10 mg / day) while taking the antioxidant drug ethoxidol allows you to achieve a comparable endothelioprotective effect of complex pharmacotherapy, which indicates the rationality of the combination of statin and antioxidant in relation to endothelioprotection.

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