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Regulation of the ratio of neurotransmitters and matrix metalloproteinases is a new strategy for secondary prevention of patients with multifocal atherosclerosis

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Multifocal atherosclerosis (MAS) remains one of the key problems of modern medicine, despite advances in primary and secondary prevention of myocardial infarction (MI) and ischemic stroke (IS). MAS worsens the prognosis and increases the risk of atherosclerotic plaque (AP) destabilization, especially under the influence of matrix metalloproteinases (MMPs). Activation of the sympatho-adrenal and serotonergic systems with the release of dopamine and serotonin also plays a significant role in the pathogenesis of this process, influencing the regulation of vascular tone and blood flow in various organs.

The aim of this study was to study the possibilities of normalizing the balance of serotonin and dopamine in patients with MAS to stabilize the atherosclerotic process and improve the regional hemodynamics of the heart, brain, and lower extremities.

Materials and methods. The study involved 54 men aged 60 to 90 years, including the first group (n = 26) patients with MAS with intermittent claudication, atherosclerotic encephalopathy and post-infarction cardiosclerosis; the second group (n = 28) patients with MAS who had IS, with concomitant atherosclerosis of the coronary and femoral arteries, and the control group – 18 people of comparable age. Examination: ankle-brachial index, Holter ECG monitoring, walking distance, dopplerography with determination of volumetric blood flow indicators. Blood levels of serotonin, dopamine, MMP-2 and MMP-9 were determined. Cognitive function was assessed using the Montreal scale. In addition, patients of both groups were prescribed cilostazol (50 mg twice a day), GABA – aminalon (250 mg twice a day) and atenolol (25 mg once a day).

Results. The results of the study showed significant disorders of hemodynamics and neurotransmitter balance in patients with MAS. We found a significant ($p < 0.001$) excess of serotonin in the blood serum, an increase of dopamine ($p < 0.01$) by 60–70 % more than in control group persons. The morning peak of a 5–7-fold increase in plasma serotonin levels is noteworthy. Levels of MMP-2 and MMP-9 were almost twice as high ($p < 0.01$) in patients who experienced IS or MI compared with the control group. After the addition of cilostazol, aminalon and atenolol to the basic therapy, we found a significant decrease in the ultra-high levels of serotonin in blood plasma ($p < 0.001$) and serum ($p < 0.05$). The levels of MMP-2 ($p < 0.01$) decreased, volumetric blood flow indicators improved in all three studied arteries ($p < 0.05$), the painless walking distance and maximum walking distance increased, and cognitive functions improved significantly ($p < 0.05$).

Conclusions. The proposed new strategy for secondary prevention of cardiovascular events in patients with MAS with a history of ischemic stroke or myocardial infarction is based on the correction of the imbalance of neurotransmitters serotonin and dopamine by adding to the basic therapy, according to the latest guidelines 2024, a complex of drugs – cilostazol, aminalon and atenolol.

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Регуляція співвідношення нейромедіаторів і матриксних металопротеїназ – нова стратегія вторинної профілактики хворих на мультифокальний атеросклероз

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Мультифокальний атеросклероз (МАС) залишається однією з ключових проблем сучасної медицини, незважаючи на досягнення в первинній і вторинній профілактиці інфаркту міокарда та ішемічного інсульту. МАС погіршує прогноз і підвищує ризик дестабілізації атеросклеротичних бляшок, особливо під впливом матриксних металопротеїназ (ММП). Активація симпато-адреналової та серотонінергічної систем із вивільненням дофаміну і серотоніну також відіграє важливу роль у патогенезі цього процесу, впливаючи на регуляцію судинного тонусу та кровотоку в різних органах.

Мета роботи – вивчити можливості нормалізації обміну серотоніну та дофаміну у хворих на МАС для стабілізації атеросклеротичного процесу і поліпшення реґіонарної гемодинаміки серця, мозку й нижніх кінцівок.

Матеріали і методи. У дослідженні взяли участь 54 чоловіки віком від 60 до 90 років. До першої групи залучили 26 хворих на МАС із переміжною кульгавістю, атеросклеротичною енцефалопатією та післяінфарктним кардіосклерозом; до другої – 28 пацієнтів із МАС, які перенесли ішемічний інсульт, із супутнім атеросклерозом коронарних і феморальних артерій. До контрольної групи залучені 18 осіб, зіставних за віком. Обстеження передбачало визначення кістково-плечового індексу, холтерівський моніторинг ЕКГ, визначення дистанції ходьби, доплерографію з визначенням показників об'ємного кровотоку. У крові визначали рівні серотоніну, дофаміну, ММП-2 та ММП-9. За допомогою Монреальської шкали оцінювали когнітивні функції. Додатково пацієнтам обох груп призначали цилостазол (50 мг двічі на добу), ГАМК – аміналон (250 мг двічі на добу) та атенолол (25 мг 1 раз на добу).

Результати. Встановили значні порушення гемодинаміки та нейромедіаторного обміну у хворих на МАС. Виявили вірогідне перевищення серотоніну в сироватці крові – майже вдвічі ($p < 0,001$), підвищення дофаміну на 60–70 % порівняно з показником контрольної групи ($p < 0,01$). Зафіксовано вранішні пікові підвищення рівнів серотоніну у плазмі крові – у 5–7 разів. Рівні ММП-2 і ММП-9 майже вдвічі вищі ($p < 0,01$) у пацієнтів, які перенесли ішемічний інсульт або інфаркт міокарда, порівняно з контрольною групою. Після додавання цилостазолу, аміналону й атенололу до базисної терапії виявили достовірне зниження надмірно високих рівнів серотоніну в плазмі ($p < 0,001$) та сироватці ($p < 0,05$) крові. Рівні ММП-2 знизилися ($p < 0,01$), показники об'ємного кровотоку покращилися в усіх трьох досліджених артеріях ($p < 0,05$). Крім того, зафіксовано збільшення безболісної дистанції ходьби, максимальної відстані ходьби та покращення когнітивних функцій.

Висновки. Запропонована стратегія вторинної профілактики серцево-судинних подій у хворих на МАС із перенесеним ішемічним інсультом або інфарктом міокарда ґрунтується на корекції дисбалансу нейромедіаторів серотоніну та дофаміну шляхом додавання до базисної терапії, згідно з останніми керівництвами (2024 року), комплексу препаратів – цилостазолу, аміналону та атенололу.

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Despite significant achievements in the field of primary and secondary prevention of myocardial infarction (MI) and ischemic stroke (IS), the problem of combined damage by the atherosclerotic process of vascular territories of the brain, heart and peripheral organs – generalized or multifocal atherosclerosis (MAS) requires further research aimed at development of methods of MAS secondary prevention [1].

The main reason for the development of acute hemodynamic disorders is the destabilization of atherosclerotic plaque (AP) [2] Ignoring the presence of ischemia in vital organs is a big mistake [3,4,5]. AP rupture occurs as a result of an increase in the destructive action of zinc-containing matrix metalloproteinases (MMPs). This becomes possible with an increase in the content of MMPs in the blood plasma and an increase in the content of MMPs activators in the blood [6,7,8,9].

A feature of ischemia of vital organs is the activation of protective and regulatory mechanisms of the cardiovascular system, both specific for each organ and characteristic for practically all organs. The latter include the sympatho-adrenal and serotonergic systems. The main mediators of these systems, dopamine (D) and serotonin (S), are involved in the regulation of blood supply, metabolism and functional activity of almost all organs and systems, both in conditions of pathology and in a healthy person [10,11].

Protecting against ischemia, each of the organs secretes S and D into the bloodstream. In this regard, plasma concentrations of S and D increase sharply [10,11]. Numerous studies show that pathologically high concentrations of S and D in the blood lead to myocardial ischemia, disturbances in central hemodynamics, heart rhythm, disorders of the gastrointestinal tract, deterioration of pulmonary blood circulation [12,13,14,15,16]. In addition, high concentrations of mediators directly and indirectly due to ischemia provoke an increase in the concentration of MMPs, as a result of which there is a real threat of rupture of APs prone to destabilization [2,8]. Secondary prevention is most challenging in patients with MAS and IS in anamnesis with accompanying coronary and femoral atherosclerosis. In such conditions, it is important to consider that the improvement of blood supply to the brain and lower extremities should not lead to an exacerbation of coronary heart disease.

Aim

The aim of this study was to study the possibilities of normalizing the balance of serotonin and dopamine in patients with MAS to stabilize the atherosclerotic process and improve the regional hemodynamics of the heart, brain, and lower extremities.

Materials and methods

The study included 54 male patients with MAS aged 60 to 90 years, average age 65.7 ± 4.8 years. Patients were divided into two groups. The MAS-1 group (n = 26) consisted of patients with multifocal atherosclerosis, which was clinically manifested by the syndrome of intermittent claudication (ICS) stage I–II according to the Fontaine–Pokrovsky classification, ischemic atherosclerotic encephalopathy, and post-infarction cardiosclerosis, patients had MI more than 1 year before inclusion in our study. The MAS-2 group (n = 28) consisted of patients with MAS who had suffered an ischemic stroke more than 12 months prior, currently had atherosclerotic lesions of the femoral arteries, with clinical manifestations of ICS, and coronary atherosclerosis, confirmed by coronary angiography, manifested by angina pectoris of II–III functional

classes. One of the conditions for the inclusion of patients with MAS in the study was the presence of a left ventricular ejection fraction (EF) greater than 45 %. The control group (CG) consisted of 18 practically healthy men, comparable in age to patients of the MAS group – aged from 40 to 70 years (62.8 ± 5.3 years). All patients were informed about the nature of the study and signed the informed consent before inclusion in the study.

Exclusion criteria: history of haemorrhagic stroke, less than 12 months before inclusion in the study IS or transient ischemic attack, life-threatening heart rhythm disorders (in particular, ventricular arrhythmias, prolongation of the Q-T interval), aneurysm of heart or aorta, heart failure IIA stage or higher (according to the classification of Strazhesko–Vasilenko), left ventricular EF less than 45 %, history of gastrointestinal or other bleeding, impaired liver and/or kidney function (creatinine clearance <30 ml/min), uncontrolled hypertension, oncological diseases.

A general clinical examination performed on all patients included determination of heart rate, systolic and diastolic blood pressure, general blood and urine analysis, biochemical blood analysis (creatinine, urea, K, Na, glucose, liver transaminases), lipid profile, electrocardiography (ECG). According to the standard method, the ankle-brachial index (ABI) was determined, and Holter ECG monitoring was performed to determine the frequency of painful and painless episodes of myocardial ischemia. With the help of dopplerography (HITACHI, ALOKA, ArriettaS70 device), the speed and volume indicators of blood flow in the arteries of the vascular territories were determined: cerebral – *a. carotis* interna (aCI), femoral – *a. femoralis* communis (aFC) and *a. tibialis* posterior (aTP); maximum systolic and volumetric blood flow velocity (FV) were determined. The state of the coronary circulation was evaluated according to the coronary angiography. The levels of neurotransmitters (S and D) in the blood serum, as well as the levels of MMP-2 and MMP-9 in the blood plasma were determined by the enzyme immunoassay method (ELISA). In addition, the levels of serotonin in the blood plasma (Sp) and in blood serum (Ss) were determined by the method of ion-exchange chromatography. Cognitive function was determined using the Montreal scale.

Patients in both groups of MAS received basic therapy, according to "2024 ACC / AHA / AACVPR / APMA / ABC / SCAI / SVM / SVN / SVS / SIR / VESS Guideline for the Management of Lower Extremity Peripheral Artery Disease" [1] and "2024 ESC Guidelines for the management of peripheral arterial and aortic diseases" [17], which included: statins, acetylsalicylic acid, angiotensin-converting enzyme inhibitors or angiotensin-II receptor blockers, calcium antagonists, β-adrenoceptor blockers. After the basic examination, the patients were additionally prescribed cilostazol (C) (50 mg twice a day), GABA – aminalon (Am) (250 mg twice a day) and, taking into account excessively high baseline levels of D in both groups, atenolol (At) was additionally prescribed (25 mg 1 time per day). Both groups were prescribed a selective beta1-adrenergic blocker – At, which does not penetrate the blood-brain barrier (BBB) and has unique structural formula, the free energy of which is able to block the catalytic centers of MMP-2 and MMP-9 [18]. CG patients were examined once.

This study complied with the ethical principles of the Helsinki Declaration of the World Medical Association of Physicians (revision 2008), ethical and moral requirements according to the Order of the Ministry of Health of Ukraine No. 281 dated November 1, 2000, including anonymity, confidentiality and charity.

Statistical analysis of the data was performed using the IBM SPSS program, version 23, R. The normality of the distribution of the obtained data was performed by the Shapiro–Wilk method. With a normal data distribution, the mean value (M) and standard error (± SD) were determined, when comparing the values, the Student's t-test was used. When the data distribution was different from the normal distribution, the Wilcoxon test was used, the median, first and third quartiles were calculated (Me (Q1; Q3); when evaluating the dynamics of indicators under the influence of treatment, the method of paired samples was used. Also, we used χ^2; univariate analysis of variance. The difference between data samples was considered significant at $p < 0.05$.

Results

Our studies have shown significant changes in the cardiovascular system in patients of both groups. The detected hemodynamically significant APs in the arteries of vascular territories – coronary, cerebral (aCI), femoral (aFC and aTP) confirmed the presence of MAS in the examined patients.

The clinical picture of patients of both groups of MAS corresponded to the localization of AP. All patients with MAS had AP in the coronary arteries, with a degree of stenosis from 30 % to 75 %, which was clinically manifested by the presence of signs of stable angina pectoris of II–III functional class; also, patients had clinical manifestations of atherosclerotic encephalopathy and intermittent claudication syndrome. The ABI index, as an indicator of peripheral atherosclerotic process presence, is noteworthy, which was less than 0.9 in all examined MAS groups and practically did not differ between the MAS-1 and MAS-2 groups, but significantly ($p < 0.01$) differed from the ABI indices of CG patients, which confirms the presence of atherosclerosis of the arteries of the lower extremities in patients of both groups of MAS *(Table 1).*

Table 2 presents indicators of central hemodynamics and bioelectric activity of the myocardium. In both groups of patients, blood pressure and heart rate are within the age norm. Attention is drawn to one of the main indicators of central hemodynamics – minute volume of blood flow – cardiac output (CO), as an indicator that combines the components of blood flow regulation, chronotropic and inotropic functions of the myocardium. In the MAS-1 group, CO was lower by 21.9 % compared to CG, and in the MAS-2 group by 24.4 % ($p < 0.01$ in both cases). Total peripheral vascular resistance was significantly ($p < 0.01$) higher in patients of both groups compared to CG patients, which indicates the generalization of the atherosclerotic process. Thus, in patients of the MAS-1 group, the level of total peripheral vascular resistance was higher by 21.0 %, compared to CG, and in patients of the MAS-2 group by 26.2 %, respectively ($p < 0.01$ in both cases).

The state of peripheral hemodynamics was assessed by FV in the studied vascular territories *(Table 3).* As can be seen from *Table 3*, the FV indicators in the MAS groups were significantly lower than in the CG, which indicates the presence of a generalized atherosclerotic process involving all three investigated vascular territories. So, FV in aCI in patients of groups MAS-1 and

Table 1. Clinical characteristics of the examined patients

Indicator, units of measurement	CG, n = 18	MAS-1, n = 26	MAS-2, n = 28
Age, years	62.8 ± 5.3	67.3 ± 3.4	65.2 ± 6.2
Men, n (%)	18 (100 %)	26 (100 %)	28 (100 %)
ICS	0	26 (100 %)	28 (100 %)
Ischemic stroke in anamnesis	0	0	28 (100 %)
Atherosclerotic encephalopathy	0	26 (100 %)	28 (100 %)
MI in anamnesis	0	26 (100 %)	0
Angina pectoris II–III functional class	0	26 (100 %)	28 (100 %)
AP in coronary arteries	0	26 (100 %)	28 (100 %)
AP in the femoral arteries	0	26 (100 %)	28 (100 %)
AP in the carotid arteries	0	26 (100 %)	28 (100 %)
Arterial hypertension	0	11 (42.3 %)	8 (28.6 %)
ABI	1.04 ± 0.05	0.67 ± 0.08**	0.65 ± 0.04***

*: p < 0.05; **: p < 0.01; ***: p < 0.001 – the difference in the values of the indicator compared to CG.

Table 2. Indicators of central hemodynamics in CG individuals and patients of groups MAS-1 and MAS-2 before and after treatment, M ± SD

Indicator, units of measurement	CG, n = 18	MAS-1, n = 26		MAS-2, n = 28	
		before treatment	after treatment	before treatment	after treatment
Heart rate, rates/min	68.7 ± 7.3	63.4 ± 4.7	65.1 ± 3.5	64.7 ± 5.2	66.2 ± 4.1
Systolic blood pressure, mm Hg	119.4 ± 5.2	129.6 ± 7.5	132.1 ± 5.6	135.4 ± 6.2*	136.5 ± 4.7
Diastolic blood pressure, mm Hg	80.9 ± 4.5	81.2 ± 5.2	84.7 ± 3.4	82.5 ± 7.3	82.3 ± 5.1
Ejection fraction of the left ventricle, %	61.4 ± 1.6	49.7 ± 1.6	50.9 ± 1.2	50.7 ± 1.4	52.6 ± 1.1
Cardiac output, ml/min	4.1 ± 0.2	3.2 ± 0.2*	3.5 ± 0.1#	3.1 ± 0.1*	3.4 ± 0.1#
Total peripheral vascular resistance, dyn.s.cm^{-5}	2117.5 ± 128.6	2562.3 ± 152.7**	2432.6 ± 124.1	2673.2 ± 128.6**	2516.3 ± 131.6

*: p < 0.05; **: p < 0.01; ***: p < 0.001 – significance of the difference in the values of indicators compared to CG; #: p < 0.05; ##: p < 0.01; ###: p < 0.001 – significance of the dynamics of indicators under the influence of treatment.

MAS-2 was lower than in CG, respectively, by 26.5 and 33.5 % (p < 0.001, in both cases). Such significant differences in patients of the MAS-2 group may indicate IS in anamnesis. The presence of ICS in patients of both groups of MAS was confirmed by low FV values in the arteries of the lower extremities. In particular, FV in aFC in groups MAS-1 and MAS-2 was, respectively, 54.0 % and 57.9 % less than in CG (p < 0.001 in both cases). Even lower FV was observed in aTP arteries, in which FV was lower in MAS-1 and MAS-2 groups, compared to CG, by 2.95 and 3.18 times, respectively (p < 0.001 in both cases).

During the initial examination, a significant increase in the levels of serotonin in the plasma and blood serum was found in the patients of the MAS groups *(Table 4)*. In 10 patients (38.4 %) of the MAS-1 group and 16 patients (57.1 %) of the MAS-2 group, the content of serotonin in blood plasma exceeded the reference values by 5–7 times. According to the literature, such an increase in the plasma serotonin level indicates the risk of development of the so-called "serotonin syndrome", which is manifested by severe arrhythmias and excessive blood pressure fluctuations – from hypotension to hypertension [12,15,16]. The average daily level of S in the blood serum of patients in the MAS groups also significantly exceeded this CG – 1.78 times in the MAS-1 group and 1.82 times in the MAS-2 group (p < 0.001 in both cases). The content of D in the blood of patients with MAS was also significantly higher than that of the control group – by 59.1 % and 77.4 %, respectively, in the MAS-1 and MAS-2 groups (p < 0.01 in all cases). Peak increases in D were observed in 11 (42.3 %) patients of the MAS-1 group and in 18 (64.3 %) patients of MAS-2. The dynamics of peak fluctuations of S and D are presented in *Figs. 1, 2* and *3*. This explains the significant fluctuations of blood pressure in patients of both groups. Strong direct correlations between S and D levels were observed: in the group of patients with MAS-2, the correlation coefficient was r = 0.890, and in the group of patients with MAS-1 it was equal to r = 0.690 (p < 0.05 in both cases).

Manifestations of MAS and the occurrence of acute blood flow disorders are largely related to the stability of AP. The main role in AP destabilization is played by MMPs, primarily MMP-2 and MMP-9, a high level of which is a recognized predictor of the risk

Table 3. Indicators of volumetric blood flow (FV, ml/min) in the studied vascular pools and their dynamics under the influence of treatment in the examined patients, Me (Q1; Q3)

Investigated vessel	CG, n = 18	MAS-1, n = 26		MAS-2 (in the injury part), n = 28	
		before treatment	after treatment	before treatment	after treatment
aCI	244.2 (184.7; 251.2)	179.5 (162.9; 219.4)***	212.3 (189.4; 234.7)#	162.3 (145.7; 183.5)***	194.6 (183.5; 241.2)#
aFC	201.5 (164.8; 226.3)	92.7 (74.6; 113.5)***	108.7 (89.7; 127.4)#	84.9 (64.8; 106.9)***	99.2 (86.5; 125.7)#
aTP	12.1 (8.4; 13.3)	4.1 (3.3; 5.8)***	6.2 (3.8; 7.2)#	3.8 (3.2; 5.3)***	5.6 (4.6; 6.8)#

*: p < 0.05; **: p < 0.01; ***: p < 0.001 – significance of the difference in the values of indicators compared to CG; #: p < 0.05; ##: p < 0.01; ###: p < 0.001 – significance of the dynamics of indicators under the influence of treatment.

Table 4. Levels of neurotransmitters and MMPs in patients of both groups, and their dynamics after treatment, Me (Q1; Q3)

Indicator, units of measurement	CG, n = 18	MAS-1, n = 26		MAS-2, n = 28	
		before treatment	after treatment	before treatment	after treatment
Serotonin (plasma), μg/ml	5.20 (3.82; 7.35)	13.58 (8.15; 20.85)***	6.82 (5.72; 9.34)###	17.13 (14.73; 20.85)***	9.21 (7.21; 10.54)###
Serotonin (serum), CU	1.14 (0.52; 1.87)	2.03 (0.87; 2.21)***	1.78 (0.68; 2.07)#	2.06 (1.96; 2.21)***	1.81 (1.75; 2.11)#
Dopamine, CU	0.93 (0.74; 1.45)	1.48 (1.21; 1.77)**	1.39 (1.08; 2.18)	1.65 (1.05; 2.43)**	1.49 (1.07; 2.31)
MMP-2, CU/mg protein	0.111 (0.107; 0.191)	0.176 (0.147; 0.182)**	0.140 (0.131; 0.162)##	0.177 (0.147; 0.182)**	0.130 (0.118; 0.157)##
MMP-9, CU/mg protein	0.107 (0.102; 0.178)	0.176 (0.170; 0.220)**	0.157 (0.145; 0.176)#	0.178 (0.170; 0.188)**	0.159 (0.149; 0.183)#

*: p < 0.05; **: p < 0.01; ***: p < 0.001 – significance of the difference in the values of indicators compared to CG; #: p < 0.05; ##: p < 0.01; ###: p < 0.001 – significance of the dynamics of indicators under the influence of treatment.

of acute coronary and cerebral circulatory disorders development. The level of MMP-2 and MMP-9 in the blood of patients with MAS significantly exceeded those of the control group: MMP-2 by 58.6 % and 59.5 %, MMP-9 by 64.5 % and 66.4 %, respectively in the groups MAS-1 and MAS-2 (p < 0.01 in all cases) *(Table 4)*. According to the literature, high MMPs levels in patients with MI and IS worsen the course of the disease and increase the risk of recurrent vascular accidents [8,19,20].

After the treatment, with C, Am, and At supplementation, along with the improvement of clinical manifestations, such as reduction of angina pectoris pain and feeling of heaviness in the lower extremities, the patients had increased walking distance, improved hemodynamic parameters and positive changes in the levels of neurotransmitters and MMPs. As can be seen from *Table 5*, according to Holter ECG monitoring data, after the treatment in the MAS-1 group, a decrease in the number of painful episodes of myocardial ischemia by 17.4 % and painless episodes of myocardial ischemia by 36.8 % was observed (p < 0.05 in both cases), in the MAS-2 group by 9.5 % and 24.6 %, respectively (p < 0.05 in both cases); with regard to the number of extrasystoles, the number of ventricular extrasystoles in the MAS-1 group decreased by 16.1 %, and in the MAS-2 group – by 12.2 %, and the number of supraventricular extrasystoles: by 15.2 % and 8.7 %, respectively (p < 0.05 in all cases).

Reduced lower limbs pain on exercise, reduced paresthesias and numbness in the lower extremities, i. e. ICS, was observed in all patients, which was reflected in the walking distance. After the treatment, painless walking distance increased by 48.8% in patients of the MAS-1 group and by 44.9 % in patients of the MAS-2 group, and maximum walking distance: by 42.7 % and 40.8 %, respectively (p < 0.001 in all cases). The data we obtained are comparable to the data in the references [1,17,21].

Cognitive function, which we assessed using the Montreal scale, also improved *(Table 5):* in patients of the MAS-1 group by 11.8 %, in the MAS-2 group – by 9.6 % (p < 0.05 in both cases), in particular by improving memory and attention.

According to the indicators of central hemodynamics, it is worth noting the acceleration of heart rate, increase of EF in patients of both groups of MAS, but these changes were not significant *(Table 2)*. CO increased by 9.4 % and 9.7 %, in groups MAS-1 and MAS-2, compared to data before treatment (p < 0.05 in both cases), respectively. ABI indicators did not change significant, compared to the data before treatment, which may indicate insufficiently long-term use of drugs.

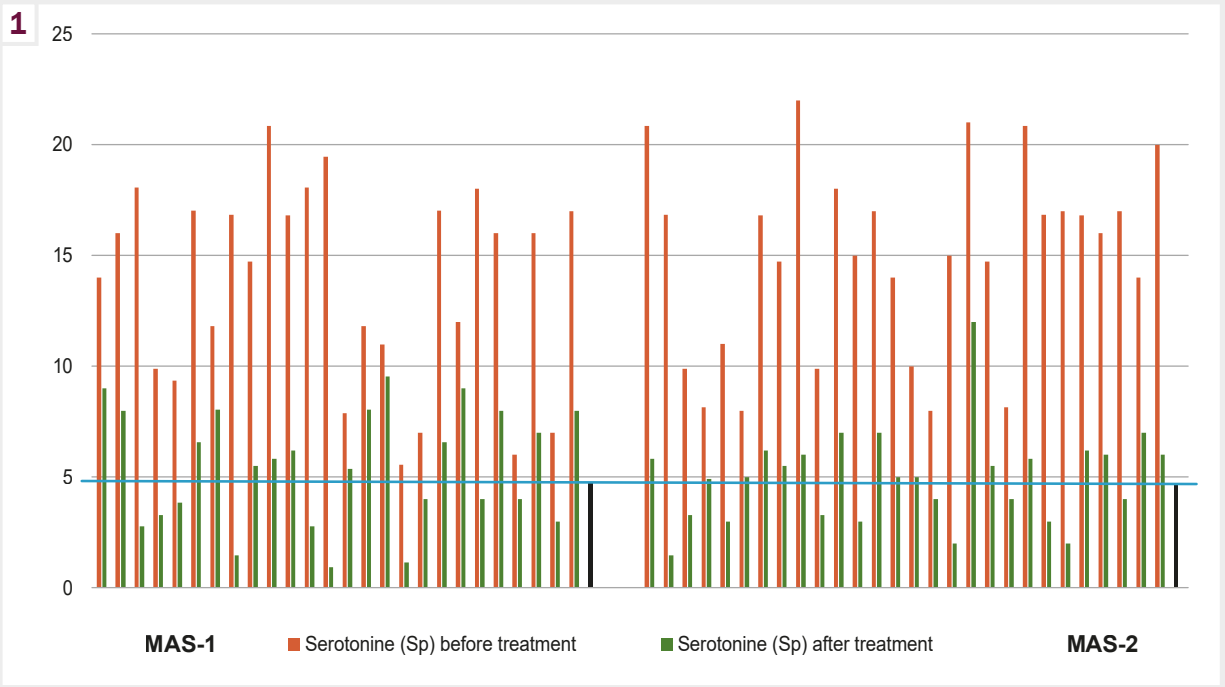

Fig. 1. Dynamics of peak morning changes in blood plasma serotonin levels (μg/ml) before and after treatment.

The horizontal line is the level of reference values of serotonin in blood plasma.

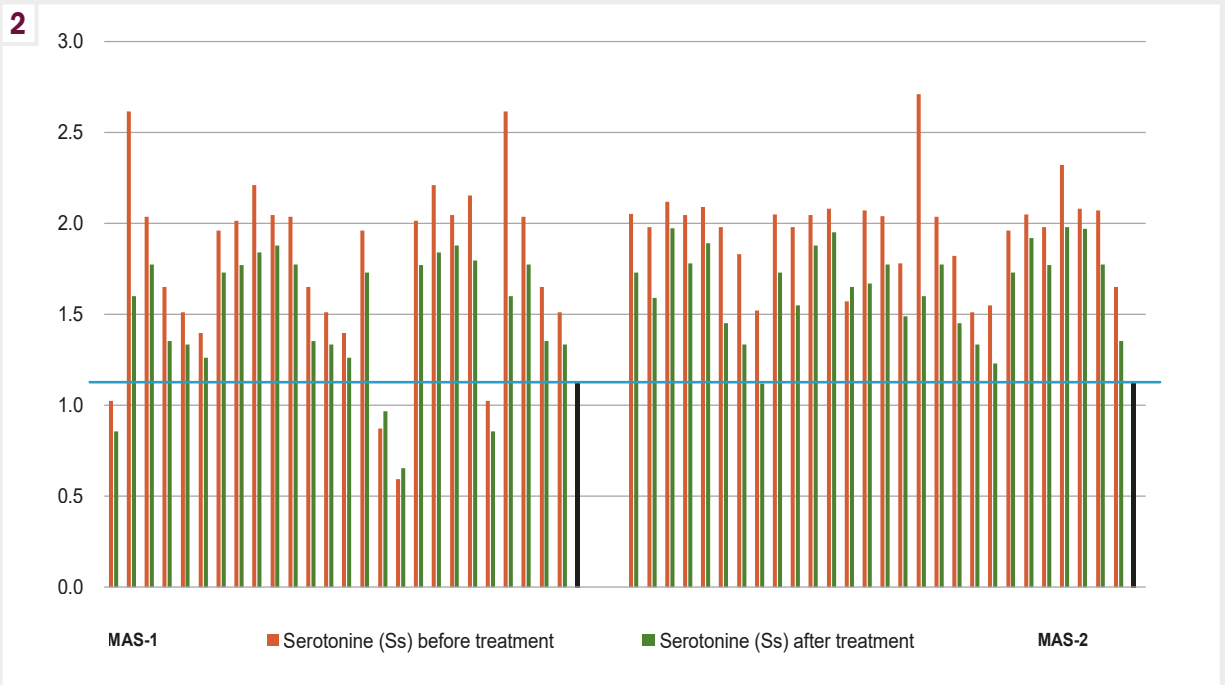

Fig. 2. Dynamics of average daily levels of serotonin in blood serum (CU) before and after treatment.

The horizontal line is the level of reference values of serotonin in blood serum.

FV indicators improved in all studied vascular territories *(Table 3)*. In patients of the MAS-1 group, FV indicators significantly increased by 18.3 % ($p < 0.05$) in aCI, by 17.3 % ($p < 0.05$) in aFC and by 51.2 % ($p < 0.01$) in aTP, in patients of the MAS-2 group by 19.9 % ($p < 0.05$); 16.9 % ($p < 0.05$); and 47.4 % ($p < 0.01$), respectively. Such a significant increase in FV indicators, in particular, in the arteries of the lower extremities, indicates a positive effect of C in patients with ICS [1].

The changes of S level in blood plasma under the influence of treatment were significant *(Table 4)*. Thus, the morning ultrahigh

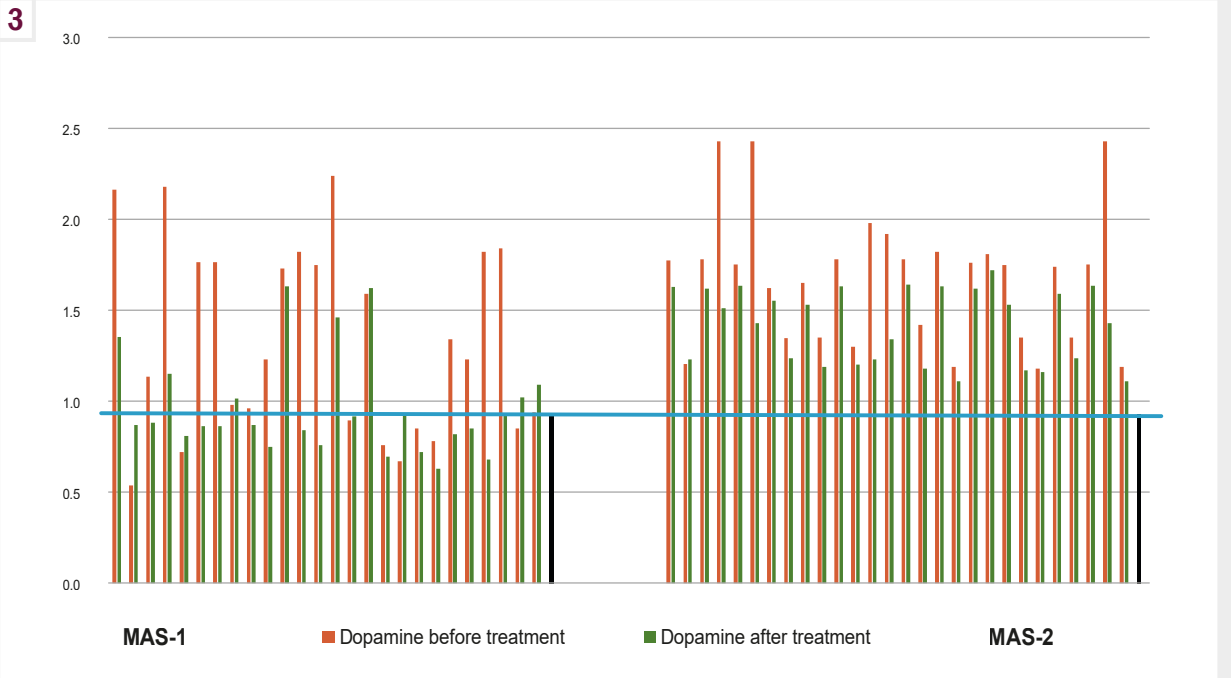

Fig. 3. Dynamics of peak morning changes in dopamine levels in blood serum (CU) before and after treatment.

The horizontal line is the level of reference values of dopamine in blood serum.

Table 5. Data of Holter ECG monitoring, walking distance and cognitive disfunction in patients before and after treatment, M ± SD

Indicator, units of measurement	MAS-1		MAS-2	
	before treatment	after treatment	before treatment	after treatment
Number of painful episodes of myocardial ischemia with ST elevation	2.3 ± 0.2	1.9 ± 0.2*	2.1 ± 0.1	1.9 ± 0.1*
Number of painful episodes of myocardial ischemia with ST depression	1.7 ± 0.2	1.2 ± 0.3*	1.3 ± 0.2	1.1 ± 0.1
Number of painless episodes of myocardial ischemia	6.8 ± 0.3	4.3 ± 0.4*	5.7 ± 0.1	4.3 ± 0.2*
Number of ventricular extrasystoles	65.4 ± 11.2	54.9 ± 8.4*	58.8 ± 9.2	51.6 ± 5.2*
Number of supraventricular extrasystoles	96.8 ± 14.7	82.1 ± 12.1*	92.6 ± 7.8	84.5 ± 8.3*
Painless walking distance, m	175.7 ± 32.4	261.5 ± 25.3***	174.2 ± 29.4	252.4 ± 22.1***
Maximum walking distance, m	372.4 ± 29.1	531.7 ± 38.4***	364.1 ± 31.2	512.8 ± 35.7***
Montreal scale of cognitive disfunction, %	20.2 ± 0.7	22.6 ± 1.1*	19.8 ± 0.9	21.7 ± 0.7*

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ – significance of the dynamics of indicators under the influence of treatment.

levels of S, exceeding reference values by 5–7 times, which were observed in 38.4 % of patients of the MAS-1 group and in 57.1 % of the MAS-2 group, decreased almost 4.6 times ($p < 0.001$ in both groups), compared to the data before treatment. Therefore, the level of Sp after additional therapy remained within the physiological range of practically healthy individuals, exceeding the reference levels of CG by only 52.4 %. Mean daily Ss levels also significantly decreased in both groups: by 12.3 % and 12.1 % ($p < 0.05$), respectively.

Regarding D levels, the changes after the treatment were not reliable, which is also a positive manifestation. Content of D were determined in blood serum, its levels exceeded the reference values almost twice. Taking into account the presence of MI and IS in the anamnesis, the use of selective beta-adrenoblockers was absolutely necessary for the purpose of cardioprotection, in this case we used atenolone (At), which does not penetrate through the BBB, therefore does not impair neuroplastic function of D in patients with IS and does not affect MMP-9, which also takes an active part in restoration of brain structure. At the same time, in the peripheral blood, the effect of D is extremely positive due to its properties of selective blockade of beta-adrenoreceptors and a decrease in the activity of MMP-2 and MMP-9, which is

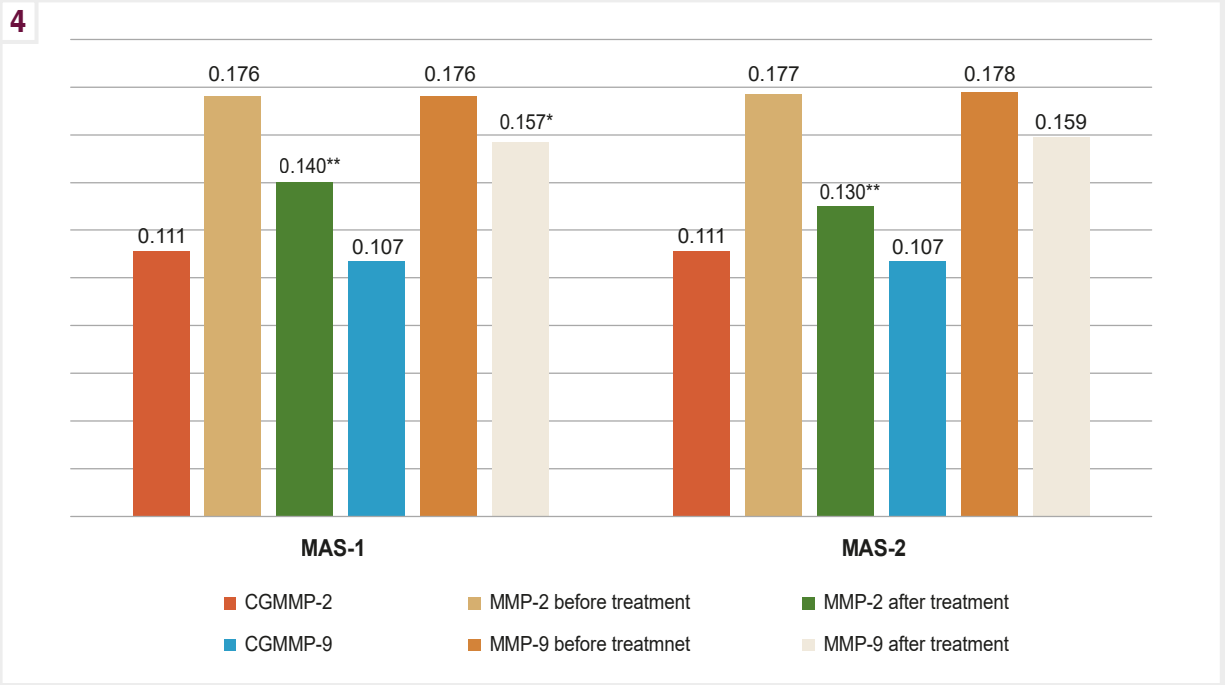

Fig. 4. Dynamics of MMP-2 and MMP-9 (CU/mg protein) levels before and after treatment.

:** $p < 0.05$; *:** $p < 0.01$ – dynamics of the indicator under the influence of treatment.

essential for the stabilization of unstable atheromatous plaques and cardioprotection [10,22,23].

Levels of MMP-2 and MMP-9 significantly decreased in both groups after additional therapy *(Fig. 4)*. The level of MMP-2 significantly decreased by 20.5 % in the MAS-1 group and by 26.6 % in the MAS-2 group ($p < 0.01$ in both cases). It should be noted that 61.5 % (16/26) of patients in the MAS-1 group and 53.6 % (15/28) in the MAS-2 group, had MMP-2 levels return to those of healthy people in the control group as a result of the treatment. In the MAS-1 group, the MMP-9 level decreased by 10.8 % ($p < 0.05$).

Discussion

We conducted a comprehensive study of patients with MAS with injury to three vascular territories: cerebral, coronary, and femoral, allowing us to identify an imbalance in the system of neurotransmitters and MMPs in patients with preserved EF. In MAS, the essence of the pathological process is the increasing ischemia of many organs, including vital ones, which ultimately leads to cardiovascular diseases, such as IS, MI and others.

Each of the organs has a complex of neurotransmitters, among which the main place is occupied by serotonin, dopamine, histamine, angiotensin, the task of which is to ensure adequate blood supply and metabolism, but because of ischemia of several organs, the level of neurotransmitters increases to critical values, which can lead to cardiovascular diseases. In this work, we consider the nature of changes in S and D levels, which when significantly increased cause pathological syndromes, such as serotonin syndrome, which can lead to death, and dopamine syndrome – increased blood pressure, tachycardia, and cardiovascular damage.

We observed a significant excess of S in the blood serum, almost twice the reference values, and an increase in D by 60–70 % compared to CG individuals. The morning peak increases of S levels by 5–7 times in blood plasma, which were revealed in 10 (38.4 %) patients of the MAS-1 group and 16 (57.1 %) patients of the MAS-2 group, compared with the reference values, are noteworthy. Excessive increase of plasma S in the morning may be one of the causes of frequent cardiovascular events, critical changes in blood pressure, heart rate, manifestations of cerebral symptoms, which can lead to repeated IS, MI or sudden coronary death. Similar increases in D levels were observed in both groups of patients.

Our studies in patients with MAS who have suffered ischemic stroke have shown a strong correlation between the concentration of S and D in peripheral blood ($r = 0.89$, $p < 0.05$). An important feature of brain tissue damage is an increase in MMPs levels both in brain tissue, cerebrospinal fluid, and peripheral blood, while an increase in MMP-9, as well as D, in the cerebrospinal fluid is a factor that contributes to the restoration of brain tissue structure and function after ischemic stroke, and high level of MMP-2 is now recognized as a predictor of the myocardial infarction development [8,19,20]. Our data indicate that the levels of MMP-2 and MMP-9 were on average 58.7 % and 64.2 % higher in patients who have suffered ischemic stroke or MI, compared with the control group.

Based on the data obtained, we formed a preliminary hypothesis about the role of neurotransmitters in the development of repeated cardiovascular events. In this regard, we have several

tasks: the first is to find ways to selectively reduce S levels, first of all the peak increase in the morning; the second is to maintain a high level of D in the CNS; the third is to eliminate the pathological effects of high D level on the cardiovascular system; the fourth is to reduce the level of MMP-2 as one of the main factors of destabilization of atherosclerotic plaques in the coronary arteries and peripheral arteries; the fifth is to improve blood supply to the brain and lower extremities.

In patients with MAS with damage to three vascular territories, there is a need to prescribe drugs that would provide increased blood supply to all three vascular territories, and ignoring one of them, in particular, the femoral, is dangerous, especially in patients with MAS. According to the literature, this can lead to rapid progression of cardiovascular diseases, with the development of recurrent cardiovascular diseases, such as MI, IS, pulmonary embolism, thrombosis of the mesenteric and renal arteries, etc. Basic therapy is based on the guidelines for the treatment of patients with previous IS, MI and peripheral arterial disease [1,17].

After extensive theoretical and clinical studies, the first task was solved by using C, to reduce the level of S, in doses half as low as the generally accepted ones: 25–50 mg (the dose was in accordance with the recommendations of geriatricians). The therapeutic effect was expressed in the elimination of the morning peak of 5–7-fold increase in serotonin levels, compared to reference values [1,24,25,26]. The use of C in patients with MAS in both groups increased the volumetric blood flow in the carotid and femoral arteries. Most importantly, C and Am significantly reduced the level of MMP-2 in patients with MAS in both groups. After taking C and At, the level of D in the peripheral blood did not change significantly. The addition of At and the replacement of previously prescribed beta-blockers with At was associated with its ability to block the active center of MMP-9. The problem with the chronotropic effect of C was solved by adding the neurotransmitter Am and At, which led to decrease the number of extrasystoles and heart rate [27].

The worldwide recognized drug for improving peripheral circulation, in particular increasing walking distance in patients with peripheral atherosclerosis, is C (Class 1A recommendation) [1], also C in combination with acetylsalicylic acid, improves secondary prevention of IS and safety outcomes for patients with lacunar infarction and does not increase the risk of severe or life-threatening bleeding [17,21]. Our studies have shown an increase in the effectiveness of C in patients with ICS stage I–II (according to the Fontaine-Pokrovsky classification) with preserved left ventricular systolic function (EF >45 %).

Side effects of C can manifest as increased heart rate, headache and sometimes arrhythmias, which can be observed when using inappropriately high doses. In our case, we considered the age of the patients, comorbidity of pathology with individual selection of the dose and its titration, this also applied to At. In our opinion, in heart failure and coronary artery disease patients require mandatory use of selective beta-blockers such as propranolone, bisoprolone, atenolol, etc. In our study, both groups of patients had clinical manifestations of coronary heart disease and coronary atherosclerosis, confirmed by coronary angiography, so our choice was At, a selective beta-blocker. The use of beta-blockers leads to an increase in beta-adrenergic receptors and this effect was constantly levelled off. In addition, of the whole group of beta-blockers, At does not penetrate the BBB and has unique structural formula, the free energy of which is capable of blocking the catalytic centers of MMP-2 and MMP-9 [18]. According to the results of our study, in 61.5 % (16/26) of patients in the MAS-1 group and in 53.6 % (15/28) of the MAS-2 group, the level of MMP-2 returned to the level of healthy people in the control group as a result of the treatment.

The second problem was in necessity to reduce excessively high levels of D in the peripheral blood, while maintaining its high concentrations in the brain, which is of key importance for the favourable course of ischemic stroke and regeneration in the affected area, as evidenced by numerous clinical studies [10,22,23]. However, excessively high levels of D may have negative consequences for the course of coronary heart disease and hypertension. We solved this problem by using the selective beta1-adrenoblocker At, which does not penetrate the BBB and simultaneously shows the ability to block beta1-adrenoreceptors and D2-dopamine receptors, while exhibiting a cardioprotective effect [13,18,23,28].

MMP-2 levels were significantly reduced in patients in both groups, indicating a reduced risk of recurrent cardiovascular events. The improvement of the clinical picture in patients was manifested in a decrease of pain in the lower extremities, which was confirmed by an increase in volumetric blood flow in the arteries of the lower extremities and an increase in painless walking distance and maximum walking distance in patients of both groups. The cardioprotective effect was represented by a decrease in the frequency of angina pectoris, a decrease in the number of extrasystoles and episodes of myocardial ischemia according to Holter monitoring. The improvement of cerebral blood supply was evidenced by an increase in volumetric blood flow in aCI and improvement of cognitive functions according to Montreal scale in patients of both groups, mainly due to memory and attention.

Conclusions

1. Patients with MAS with an injury of cerebral, coronary and femoral vascular territories, clinically manifested by ischemic stroke and myocardial infarction, intermittent claudication, had pathologically high blood plasma levels of serotonin, dopamine, MMP-2 and MMP-9, high level of which is associated with the risk of cardiovascular events development.

2. The use of the presented drug combination (cilostazol, aminalon, atenolol) allowed to reduce peak morning increases of serotonin and dopamine due to the effect of cilostazol and increase of volumetric blood flow in the examined arteries. The undesirable side effect of cilostazol in the form of increased heart rate was counteracted by the action of aminalon and atenolol.

3. The suppression of the pathological effect of MMP-2 and MMP-9 was carried out due to the action of cilostazol, which significantly reduced their levels, as well as the unique property of atenolol, which inhibits the active centers of MMPs, reducing their activity. Thus, the combination of cilostazol and atenolol not only reduced the level of MMPs but also inhibited their activity.

4. Our proposed new strategy of secondary prevention of cardiovascular events in patients with MAS with ischemic stroke or myocardial infarction is based on the correction of imbalance of neurotransmitters, such as serotonin and dopamine, by adding a complex of drugs – cilostazol, aminalon and atenolol – to the basic therapy, according to the latest guidelines.

Prospects for further research. Disadvantages of the presented work: the research was conducted on a small cohort of patients, data were provided on only 2 neurotransmitters (serotonin, dopamine), we are currently continuing studies on serotonin, dopamine, histamine, angiotensin, which will be presented in subsequent publications after the end of the research.

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