PRODUCTION OF ANGIOGENESIS MEDIATORS AND THE STRUCTURE OF THE VASCULAR WALL IN THE HEART IN ISCHEMIC CARDIOMYOPATHY

Chumakova S.P. [1],
Urazova O.I. [1],
Shipulin V.M. [1],
Sukhodolo I.V. [2],
Stelmashenko A.I. [1],
Denisenko O.A. [1],
Andreev S.L. [2],
Demin M.S. [1],
Churina E.G. [1, 3]

[1] Siberian State Medical University (Moskovsky Trakt 2, Tomsk 634050, Russian Federation)
[2] Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences (Kievskaya str. 111A, Tomsk 634012, Russian Federation)
[3] National Research Tomsk State University (Lenina Ave. 36, Tomsk 634050, Russian Federation)

Corresponding author:
Olga A. Denisenko,
e-mail: olga-muraveinik@yandex.ru

ABSTRACT

Background. *In the pathogenesis of ischemic cardiomyopathy (ICMP), angiopoiesis remains unexplored.*
The aim of the study. *To describe the vasculature of the heart and the imbalance of angiogenesis mediators in the coronary circulation in association with the number of endothelial progenitor cells (EPC) and desquamated endothelial cells (DEC) in the blood of patients with coronary heart disease (CHD), suffering and not suffering from ICMP.*
Materials and methods. *Fifty-two patients with CHD (30 patients with ICMP, 22 patients without ICMP), 15 healthy donors were examined. The content of EPC ($CD14^+CD34^+VEGFR2^+$) in the blood from the cubital vein and DEC ($CD45^-CD146^+$) in the blood from the coronary sinus and the cubital vein was determined by flow cytometry. The concentrations of VEGF-A (vascular endothelial growth factor A), PDGF (platelet-derived growth factor), and SDF-1 (stromal cell-derived factor 1) in blood plasma were recorded using immunofluorescence assay; the angiopoietin-2, MMP-9 (matrix metallopeptidase 9) were recorded using enzyme immunoassay. In myocardial biopsies the specific area of vessels and the expression of αSMA (smooth muscle alpha-actin) were determined by morphometric and immunohistochemical methods.*
Results. *In the peripheral blood of patients with CHD, regardless of the presence of ICMP, the DEC content exceeded the physiological level, and the VEGF-A, PDGF, angiopoietin-2, and MMP-9 corresponded to the norm. In CHD patients without cardiomyopathy, there was an excess of SDF-1 and EPC in the blood from the cubital vein, and in ICMP, their physiological significance was noted. In the coronary blood flow in patients with CHD without cardiomyopathy, an increase in the concentration of PDGF was found, which was not determined in patients with ICMP, who had an increased content of DEC, angiopoietin-2 and MMP-9. The specific area of the vessels in the patients of the two groups was comparable; the expression of αSMA in ICMP was 6.2 times lower than in patients with CHD without cardiomyopathy.*
Conclusion. *The development of ICMP is accompanied by impaired maturation of vessels in the myocardium, associated with the absence of a compensatory reaction of activation of cellular and humoral factors of angiogenesis.*

Key words: *angiogenesis, growth factors, endothelial progenitor cells, myocardium, coronary heart disease*

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ПРОДУКЦИЯ МЕДИАТОРОВ АНГИОГЕНЕЗА И СТРУКТУРА СОСУДИСТОЙ СТЕНКИ В СЕРДЦЕ ПРИ ИШЕМИЧЕСКОЙ КАРДИОМИОПАТИИ

Чумакова С.П. [1],
Уразова О.И. [1],
Шипулин В.М. [2],
Суходоло И.В. [1],
Стельмашенко А.И. [1],
Денисенко О.А. [1],
Андреев С.Л. [2],
Дёмин М.С. [1],
Чурина Е.Г. [1, 3]

[1] ФГБОУ ВО «Сибирский государственный медицинский университет» Минздрава России (634050, г. Томск, Московский тракт, 2, Россия)
[2] Научно-исследовательский институт кардиологии, ФГБУ «Томский национальный исследовательский медицинский центр Российской академии наук» (634012, г. Томск, ул. Киевская, 111а, Россия)
[3] ФГАОУ ВО «Национальный исследовательский Томский государственный университет» (634050, г. Томск, просп. Ленина, 36, Россия)

Автор, ответственный за переписку:
Денисенко Ольга Анатольевна,
e-mail: olga-muraveinik@yandex.ru

РЕЗЮМЕ

Актуальность. *При ишемической кардиомиопатии (ИКМП) ангиогенез остаётся неизученным.*
Цель исследования. *Охарактеризовать сосудистую сеть сердца и дисбаланс медиаторов ангиогенеза в коронарном кровотоке в ассоциации с численностью эндотелиальных прогениторных клеток (ЭПК) и десквамированных эндотелиальных клеток (ДЭК) в крови у больных ишемической болезнью сердца (ИБС), страдающих и не страдающих ишемической кардиомиопатией.*
Методы. *Обследованы 52 больных ИБС (30 пациентов с ИКМП, 22 пациента без ИКМП) и 15 здоровых доноров. В крови из кубитальной вены определяли содержание ЭПК ($CD14^+CD34^+VEGFR2^+$), из коронарного синуса и кубитальной вены – ДЭК ($CD45^-CD146^+$) методом проточной цитофлуориметрии. В плазме крови регистрировали концентрацию фактора роста эндотелия сосудов A (VEGF-A, vascular endothelial growth factor A), фактора роста тромбоцитов (PDGF, platelet-derived growth factor), стромального клеточного фактора 1 (SDF-1, stromal cell-derived factor 1) с помощью иммунофлуоресцентного анализа; ангиопоэтина-2, матриксной металлопротеиназы 9 (MMP-9, matrix metallopeptidase 9) – методом иммуноферментного анализа. В биоптатах миокарда определяли удельную площадь сосудов и экспрессию αSMA (smooth muscle alpha-actin) морфометрическим и иммуногистохимическим методами.*
Результаты. *В периферической крови у больных ИБС вне зависимости от наличия ИКМП содержание ДЭК превышало физиологический уровень, а содержание VEGF-A, PDGF, ангиопоэтина-2 и MMP-9 соответствовало норме. У больных ИБС без кардиомиопатии в крови из кубитальной вены отмечался избыток SDF-1 и ЭПК, а при ИКМП – их физиологическое значение. В коронарном кровотоке у больных ИБС без кардиомиопатии установлено повышение концентрации PDGF, чего не определялось у пациентов с ИКМП, у которых было увеличено содержание ДЭК, ангиопоэтина-2 и MMP-9. Удельная площадь сосудов у больных двух групп была сопоставимой, экспрессия αSMA при ИКМП была в 6,2 раза ниже, чем у больных ИБС без кардиомиопатии.*
Заключение. *Развитие ИКМП сопровождается нарушением созревания сосудов в миокарде, связанным с отсутствием компенсаторной реакции активации клеточных и гуморальных факторов ангиогенеза.*

Ключевые слова: *ангиогенез, факторы роста, эндотелиальные прогениторные клетки, миокард, ишемическая болезнь сердца*

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INTRODUCTION

Ischemic cardiomyopathy (ICMP) is a severe disease that has no specific pharmacotherapy to date and is characterised by disease progression in some patients even after surgical correction of the coronary bed and left ventricular cavity [1, 2]. This demonstrates the insufficiently studied pathogenesis of ICMP, in which the role of chronic inflammation, cardiomyocyte apoptosis, disorders of Ca^{2+} homeostasis and myocardial contractile function, synthesis of different types of collagens, and microvascular dysfunction have been actively discussed to date [2–4]. Therewith, the interest of scientists is focused on the vasomotor form of endothelial dysfunction [5, 6]. Angiogenic form of endothelial dysfunction in ICMP, including impaired angiogenesis, balance of reparative and destructive processes in vessels [7], however, has not been studied.

Both forms of chronic coronary heart disease (CHD) are accompanied by damage to the vascular intima, since the morphological substrate of CHD, either complicated or uncomplicated by ICMP, is atherosclerosis of the coronary arteries. On the one hand, plaque macrophages support chronic inflammation, prolong vascular alteration and endothelial desquamation with the help of matrix metalloproteinases (MMP) [1, 8, 9], but they also contribute to atheroma vascularisation, which increases the risk of plaque haemorrhage with its subsequent destabilisation [5, 10]. On the other hand, induction of angiogenesis is necessary for formation of collateral blood flow and repair of damaged vessels, which has protective and adaptive value in CHD and ICMP. Angiogenesis is performed by endothelial progenitor cells (EPC), most of which have a monocytic immunophenotype and reparative potential in relation to endothelium as a result of paracrine secretion of angiogenesis factors [11].

In this regard, studying the output of such mediators of angiogenesis as vascular endothelial growth factor (VEGF) A, platelet-derived growth factor (PDGF), stromal cell-derived factor (SDF) 1, angiopoietin (Ang) 2 and MMP-9 in the heart [11, 12] can establish the mechanisms of angiogenesis and angiopoietic endothelial dysfunction in CHD both complicated and uncomplicated by ICMP. At the same time, comparison of the number of EPCs of monocytic immunophenotype and desquamated endothelial cells (DEC) in blood, as well as determination of the specific volume of vessels and expression of alpha-smooth muscle actin (αSMA), which is synthesized by vascular smooth muscle cells [13], will enable to determine the correlation of angiogenesis factors with the degree of coronary endothelial damage in ICMP relative to CHD without cardiomyopathy.

THE AIM OF THE STUDY

To reveal features of vascular network formation in the heart and imbalance of angiogenesis mediators in coronary blood flow in association with the number of endothelial progenitor and desquamated cells in blood from patients affected by coronary heart disease with and without ischemic cardiomyopathy.

METHODS

A single-stage controlled (case-control) single-center observational study was conducted from February 2020 to May 2022. The study included 52 CHD patients with tension angina II–IV functional class and circulatory insufficiency, mainly II–III functional class according to NYHA (New York Heart Association), who had a history of myocardial infarction and were hospitalised at the Research Institute of Cardiology of Tomsk National Research Medical Centre of the Russian Academy of Sciences for the purpose of coronary bypass surgery. Patients with CHD were categorised into two groups: 30 patients with ICMP (27 men and 3 women; mean age – 61.0 [56.0; 64.0] years) and 22 patients without cardiomyopathy (18 men and 4 women; mean age – 64.0 [59.5; 67.0] years). According to the criteria of G.M. Felker et al. (2002), the signs of ICMP were low left ventricular ejection fraction (less than 40 %), haemodynamically significant stenosis of two or more epicardial vessels or the trunk of the left descending artery [14]. CHD patients without cardiomyopathy had similar coronary vessel changes but had preserved left ventricular ejection fraction (more than 40 %). The control group consisted of 15 practically healthy donors (13 men and 2 women; mean age – 57.63 ± 8.12 years) without any cardiovascular diseases and relevant complaints.

CHD patients both with and without ICMP were comparable in terms of age, sex, body mass index, duration of CHD, functional class of angina and circulatory insufficiency, as well as frequency of prescription of statins. However, they were statistically significantly different in terms of left ventricular parameters: ICMP patients compared to CHD patients without cardiomyopathy had higher myocardial mass (233.0 [221.7; 266.2] g vs. 184.0 [140.5; 214.5] g; $p < 0.001$) but lower ejection fraction (30.00 [22.00; 36.00] % vs. 59.50 [50.25; 67.00] %; $p < 0.001$), as a decrease in the latter less than 40 % was a criterion for ICMP diagnosis and patient grouping. The pattern of comorbidity in the patient cohorts was also comparable, except for a higher incidence of type 2 diabetes mellitus in CHD patients without ICMP (31.82 % vs. 6.67 %; $p = 0.046$) and chronic cerebral circulatory disorders in patients with ICMP (90.0 % vs. 59.1 %; $p = 0.023$).

All CHD patients underwent coronary artery bypass surgery with similar anaesthetic management (diazepam, ketamine, fentanyl, promedol, pipecuronium). At the preoperative stage, patients of both groups were treated according to the generally accepted principles of CHD therapy (prolonged-acting nitrates and on demand – calcium channel blockers, β1-adrenoblockers, statins, antiaggregants). Therapy was similar in the CHD patient groups, except for more frequent use of calcium channel blockers in CHD patients without cardiomyopathy, compared with ICMP patients (63.6 % vs. 0 %; $p < 0.001$). More frequent prescription of anticoagulants in CHD patients without cardiomyopathy may

be associated with a greater intensity of atherogenesis and involvement of lower limb vessels than in ICMP.

The exclusion criteria of patients from the study were as follows: age older than 70 years; presence of allergic disease in the exacerbation stage, autoimmune diseases, anaemia, tumour process, syphilis, HIV infection, viral hepatitis; presence of acute infectious diseases less than 3 weeks before surgery; prescription of erythropoietin or immunosuppressive therapy; patient's refusal to participate in the study.

The studies were conducted in accordance with the ethical principles outlined in the World Medical Association Declaration of Helsinki (1975) and with the permission of the local Ethical Committee of the Siberian State Medical University of the Ministry of Health of Russia (Protocol No. 7981 dated December 16, 2019). Informed consent for participation in the study was obtained from all individuals examined.

The study material included blood samples from the cubital vein (peripheral blood) and blood from the coronary sinus (sinus blood) stabilized with heparin (25 IU/ml), as well as biopsy specimens of the auricle of the right atrium. Peripheral blood was collected in a volume of 5 ml from the cubital vein in the morning on an empty stomach in both healthy donors and CHD patients of both study groups on the day of surgery immediately before induction into anaesthesia. Peripheral blood was used for immunophenotyping of EPCs and DECs, its plasma was used to estimate the concentration of the studied mediators. Blood from the coronary sinus in the volume of 5 ml was obtained only in CHD patients: intraoperatively, by transmyocardial puncture after surgical access to the heart, but before connection of the artificial circulation device and the main stage of the surgery. DEC content was determined in blood from the coronary sinus, blood plasma from the coronary sinus was used to study the concentration of the studied mediators. Myocardial biopsies of the right atrial auricle in volume not more than 10 mm^3 were obtained intraoperatively at the stage of its cannulation for connection of the artificial circulation device, but before the commencement of extracorporeal perfusion. Myocardial biopsy specimens were used to determine specific vessel area by morphometric method and α-SMA expression by immunohistochemical method.

DEC absolute amount and EPC relative content in blood were determined by flow cytofluorimetry in venous blood obtained from the cubital vein in healthy donors and in CHD patients of both groups (peripheral blood). In patients, DEC content was also assessed in blood from the coronary sinus. Whole blood was lysed by adding FACS Lysing solution (BD Biosciences, USA), then cells were washed three times with 20-fold volume of Cell-WASH-solution BD buffer (Becton Dickinson, USA). Mouse Anti-Human CD14-FITC, CD34-PE, VEGFR2(KDR; CD309)-Alexa Fluor 647, CD45-FITC and CD146-Alexa Fluor 647 monoclonal antibodies were used to detect EPCs with $CD14^+CD34^+VEGFR2^+$ immunophenotype and DECs with $CD45^-CD146^+$ immunophenotype, according to the manufacturer's instructions (BD Biosciences, USA). Fluorescence intensity measurements were performed using an Accuri C6 flow cytometer (BD Biosciences, USA), and the data were analyzed using BD Cell Quest for Mac OS® X software application (BD Biosciences, USA). DEC fraction among all blood cells analysed was correlated with the total number of leukocytes expressing $CD45^+$ (CD45 – total leukocyte antigen), expressed in $\times 10^5$/l. The total number of leukocytes in blood was assessed by flow cytofluorimetry using a XS-1000i haematological analyzer (Sysmix Corporation, Japan).

Peripheral blood plasma from CHD patients of both study groups and healthy donors, as well as blood plasma from the coronary sinus of CHD patients of both groups was aliquoted and stored at –80 °C for no more than 12 months. The concentration of VEGF-A, PDGF, SDF-1 was measured using a commercial multiplex assay test system 'Magnetic Luminex Assay Kit for VEGFA, VEGFB, PDGF, SDF1, SCF, FGF1, GM-CSF, MSR1' (Cloud-Clone Corp., USA) and an automated analyzer Bio-Plex Protein Assay System (Bio-Rad, USA). The concentration of Ang-2 and MMP-9 proteinase in plasma was measured by enzyme-linked immunosorbent assay using commercial kits 'RayBio Human ANGPT2 ELISA Kit' (RayBiotech, USA) and 'Human MMP9 ELISA' (ThermoFisher Scientific, USA) according to the manufacturers' instructions.

The myocardial samples obtained were fixed in 10 % neutral buffered formalin, paraffinized and histological sections 4–5 μm thick were made using an automatic rotary microtome HM 355 S (Thermo Scientific, USA). Sections were stained with hematoxylin and eosin [15], enclosed in BioMount mounting medium (BioOptica, Italy). Immunohistochemical staining was performed on 4 μm thick paraffin sections for which deparaffinization, antigen demasking, and blocking of non-specific binding with 3 % bovine serum albumin in phosphate-buffered saline (PBS) were performed. Following this, slices were incubated with primary antibodies to αSMA (Spring BioScience, USA) for 60 min in a humid chamber followed by 3-fold washing in PBS, then incubated with secondary HRP-labelled antibodies for 45 min followed by 3-fold washing in PBS. In the last step, DAB-chromagen substrate (HRP-DAB (horseradish peroxidase – diaminobenzidine) imaging system; DAKO, USA) was added and stained with haematoxylin. All sections were enclosed in BioMount mounting medium (BioOptica, Italy). The preparations were studied in transmitted light using an Axioskop 40 microscope (Carl Zeiss, Germany); images were digitized using a Canon G 10 camera (Canon, Japan). Tissue markers were counted at ×400 magnification in 10 randomly selected fields of view corresponding to 1 mm^2 of tissue [16]. Specific vessel area and αSMA expression as percentage of the studied tissue area were estimated using the AxioVision graphic image processing program (Carl Zeiss, ImageJ).

Statistical analysis of the data was performed using Statistica 10.0 program (StatSoft Inc., USA). In statistical description of the results, median, 25th and 75th percentiles were calculated for quantitative traits; for qualitative traits, sample fraction was estimated. In order to comparatively analyze sample data, Mann – Whitney (for independent samples) and Wilcoxon (for dependent samples)

criteria were applied, using Benjamini – Hochberg correction for multiple comparisons. Chi-square test with Yeats' correction for continuity was applied to compare the frequencies of occurrence of the trait in the groups. The results of statistical analysis were considered statistically significant at $p < 0.05$.

RESULTS

The DEC content in peripheral blood of CHD patients, whether ICMP was present or not, was higher than that of healthy donors and did not differ between patient groups in both blood from the cubital vein (Table 1) and blood from the coronary sinus (Table 2). At the same time, EPC abundance in peripheral blood was elevated in CHD patients without cardiomyopathy (Table 1). In patients with ICMP, on the contrary, this parameter of total blood flow varied within physiological values (Table 1), while DEC abundance in sinus blood was 2.5 times higher than in peripheral blood, which was not observed in CHD patients without cardiomyopathy (Tables 1, 2).

The content of VEGF-A and PDGF growth factors in peripheral blood of CHD patients corresponded to the values in healthy donors irrespective of ICMP presence and did not differ between the patient groups (Table 1), but the coronary blood flow analysis revealed significant differences (Table 2). For instance, in CHD patients without cardiomyopathy, PDGF levels were higher in sinus blood than in peripheral blood (Tables 1, 2). Meanwhile, VEGF-A content in blood from the coronary sinus prevailed over its level in blood from the cubital

TABLE 1

CONTENT OF ENDOTHELIAL PROGENITOR AND DESQUAMATED CELLS AS WELL AS MEDIATORS OF ANGIOGENESIS IN BLOOD FROM THE CUBITAL VEIN IN CHD PATIENTS AFFECTED AND NOT AFFECTED BY ICMP, Me [Q1; Q3]

Indicators	Group of examined individuals		
	CHD without ICMP	CHD with ICMP	Healthy donors
EPC content $VEGFR2^+CD34^+CD14^+$, %	0.74 [0.46; 1.23] $\boldsymbol{p_k < 0.001}$	0.31 [0.15; 0.64] $p_k = 0.260$ $\boldsymbol{p = 0.038}$	0.19 [0.13; 0.32]
DECs number $CD45^-CD146^+$, $\times 10^5$/l	7.25 [6.80; 7.47] $\boldsymbol{p_k = 0.038}$	7.26 [5.43; 17.94] $\boldsymbol{p_k = 0.037}$ $p = 0.597$	5.12 [3.73; 5.84]
VEGF-A, pg/ml	4.50 [3.00; 8.00] $p_k = 0.314$	6.00 [3.00; 9.50] $p_k = 0.216$ $p = 0.502$	3.80 [1.00; 6.50]
SDF-1, pg/ml	60.00 [50.00; 80.00] $\boldsymbol{p_k = 0.042}$	49.00 [37.00; 56.00] $p_k = 0.174$ $p = 0.115$	30.00 [5.00; 45.00]
PDGF, pg/ml	3.10 [2.10; 7.05] $p_k = 1.000$	4.85 [1.20; 9.10] $p_k = 1.000$ $p = 0.870$	2.68 [1.65; 7.10]
Angiopoetin-2, pg/ml	445.0 [137.5; 552.5] $p_k = 1.000$	540.0 [403.0; 670.0] $p_k = 0.612$ $p = \mathbf{0}.884$	388.0 [317.0; 460.0]
MMP-9, pg/ml	11.95 [7.00; 13.40] $p_k = 0.460$	13.65 [6.50; 19.60] $p_k = 0.848$ $p = 0.588$	13.20 [9.60; 19.00]

Note. p_k – level of statistical significance of the differences in indicators compared with the content of cytokines/cells in healthy donors; p – level of statistical significance of the differences in indicators compared with the content of cytokines/cells in CHD patients without cardiomyopathy; statistically significant differences are marked in bold.

TABLE 2

DESQUAMATED ENDOTHELIAL CELLS AND MEDIATORS OF ANGIOGENESIS IN BLOOD FROM THE CORONARY SINUS IN ASSOCIATION WITH SPECIFIC VESSEL AREA CHARACTERIZATION AND ASMA EXPRESSION IN MYOCARDIUM FROM CHD PATIENTS BOTH AFFECTED AND NOT AFFECTED BY ICMP, Me [Q1; Q3]

Indicators	Group of examined individuals	
	CHD without ICMP	CHD with ICMP
DECs number $CD45^{-}CD146^{+}$, $\times 10^{5}$/l	10.17 [6.80; 18.83] $p_1 = 0.128$	17.98 [10.27; 22.97] **$p_1 = 0.036$** $p = 0.156$
VEGF-A, pg/ml	7.80 [3.25; 9.75] **$p_1 = 0.041$**	6.89 [3.25; 15.60] **$p_1 = 0.007$** $p = 0.918$
SDF-1, pg/ml	40.30 [26.00; 62.00] **$p_1 = 0.086$**	46.80 [32.50; 64.00] $p_1 = 0.286$ $p = 0.623$
PDGF, pg/ml	7.60 [3.70; 9.94] **$p_1 = 0.036$**	7.86 [2.92; 8.77] $p_1 = 0.674$ $p = 0.736$
Angiopoietin-2, pg/ml	767.0 [494.0; 988.0] $p_1 = 0.128$	1111.5 [845.0; 1235.0] **$p_1 < 0.001$** **$p = 0.002$**
MMP-9, pg/ml	5.92 [5.07; 17.42] $p_1 = 0.972$	16.64 [6.63; 29.12] $p_1 = 0.649$ **$p = 0.038$**
Vessel specific area, %	5.70 [5.60; 6.70]	6.60 [4.60; 8.90] $p = 0.815$
αSMA expression, %	8.10 [7.60; 11.30]	1.30 [0.60; 2.80] **$p = 0.007$**

Note. p_1 – level of statistical significance of the differences in indicators compared with the content of cytokines/cells in peripheral blood; p – level of statistical significance of the differences in indicators compared with the content of cytokines/cells in CHD patients without cardiomyopathy; statistically significant differences are marked in bold.

vein in CHD patients of both groups without differences between patient cohorts (Tables 1, 2). SDF-1 concentrations in peripheral blood exceeded the norm only in CHD patients without cardiomyopathy (Table 1); however, regardless of the ICMP occurrence, the level of this mediator corresponded to that in sinus blood, and no differences between the patient groups were revealed in both blood samples (Tables 1, 2).

The content of Ang-2 and MMP-9 in peripheral blood of CHD patients affected and not affected by ICMP was registered at the level of parameters of healthy donors and did not reveal any differences between patient groups (Table 1). Meanwhile, the concentration of both mediators in blood from the coronary sinus was higher in patients with ICMP than in CHD patients without cardiomyopathy (Table 2). Furthermore, the concentration of Ang-2 in sinus blood prevailed over that in peripheral blood only in patients with ICMP, and the content of MMP-9 corresponded to its content in peripheral blood independently from the ICMP occurrence (Tables 1, 2).

The study of histological preparations of myocardium revealed that the vessel specific area in CHD patients of both study groups was determined at a comparable level, but αSMA expression in patients with ICMP was 6.2 times lower than in CHD patients without cardiomyopathy (Table 2).

DISCUSSION

The obtained data demonstrates significant differences in the mediator profile of blood from the coronary sinus in CHD patients affected and not affected by ICMP, which does not correspond to the nature of the imbalance of angiogenesis factors in peripheral blood (Tables 1, 2), indicating the involvement of different mechanisms of angiogenesis regulation in the affected heart and at the systemic level. Specifically, in patients with ICMP, DEC content in blood from the coronary sinus was higher than in peripheral blood (Tables 1, 2), and EPC level in blood from the cubital vein remained normal (Table 2). In contrast, in CHD patients without cardiomyopathy, DEC abundance in blood samples was comparable (Tables 1, 2) with high levels of EPCs in the systemic bloodstream (Table 2). This indicates an increased attraction of EPCs with reparative potential from bone marrow into blood in CHD patients without cardiomyopathy, which is a compensatory reaction during atherogenesis and, obviously, provides reparative angiogenesis adequate to endothelial destruction in the heart. In ICMP patients this compensatory reaction, apparently, is not realized: physiological level of EPC in blood is insufficient for coronary vessels repair in conditions of atherosclerosis, therefore angiogenesis is not effective, and endothelial destruction prevails, which proves the presence of angiopoietic endothelial dysfunction in ICMP. Significantly, no increased destruction of coronary endothelium was revealed by measurement of DEC content in blood from the cubital vein in ICMP (Table 1).

HIF-1 (hypoxia-inducible factor 1) is a central regulator of angiogenesis as it enhances gene transcription of several pro-angiogenic proteins (SDF-1, VEGF, PDGFB, Ang-1, Ang-2) and their receptors [17], thereby preventing myocardial ischaemic damage [18]. Insufficient coronary vascular repair in ICMP may be associated with an imbalance of angiogenesis mediators that ensure EPC mobilization from the bone marrow, their homing and proliferation/differentiation/secretory activity in coronary vessels. Among the studied mediators of angiogenesis in the peripheral blood of CHD patients without cardiomyopathy manifesting EPC excess, only SDF-1 concentration was elevated, while in patients with ICMP both indices (EPC and SDF-1 content) were in compliance with the norm (Table 1). Accumulation of SDF-1 in plasma stimulates mobilization of $CXCR4^+$ cells from the bone marrow, including haematopoietic stem cells and EPCs, which express CXCR4 as a receptor for SDF-1. The interaction between SDF-1 and CXCR4 also stimulates the recruitment and retention of stem cells in ischaemic areas [12, 19].

The content of another angiogenesis activator VEGF-A in blood from the coronary sinus exceeded that in peripheral blood among CHD patients of both groups (Tables 1, 2), apparently reflecting the induction of angiogenesis under ischaemic conditions. VEGF-A binds to VEGFR1 and VEGFR2, stimulating proliferation and differentiation of EPCs into endothelial cells, formation of tubular structures and increased permeability of the vascular wall, and inhibits cardiomyocyte apoptosis [12, 20–22]. Since hypoxia increases the expression of VEGFR1 [21], which is a trap receptor for VEGF-A and can inhibit angiogenesis [22] and activate MMP-9 secretion from vascular myocytes [23], the interaction of VEGF-A with VEGFR1 may be enhanced in ICMP patients considering widespread myocardial ischaemia, explaining the lack of increase in its blood concentration. In addition to the proangiogenic VEGF-Axxxa family, there is also a family of VEGF-Axxxb isoforms that inhibit angiogenesis [23]. The synthesis of the latter increases under the action of transforming growth factor (TGF) β [23], which is actively secreted in the myocardium of ICMP patients [24]. Additionally, VEGF-A has proatherogenic properties (accumulates triacylglycerols, inhibits lipoprotein lipase), in contrast to VEGF-B, which is characterised by hypolipidemic effects [22].

Along with that, the increase of PDGF concentration in sinus blood relative to peripheral blood in CHD patients without cardiomyopathy (Tables 1, 2) indicates stabilization of newly formed vessels in the heart with VEGF-A participation, which probably does not occur in ICMP patients. PDGF is known to promote not only differentiation, mobilization of EPCs from bone marrow and their migration [25], but also vascular maturation since, unlike VEGF, it attracts pericytes [26], vascular smooth muscle cells and stimulates endothelial-mesenchymal transition [27]. It is being activated in the vascular wall and represents the process of loss of EPC endothelial phenotype and their transdifferentiation into smooth muscle cells, but in dilated cardiomyopathy it is also accompanied by the transition of EPCs into myofibroblasts [28]. PDGF addition to smooth muscle cell culture *in vitro* increases their survival through activation of a signalling pathway involving Notch3, and stimulation of Notch1 signalling maintains their contractile phenotype [13]. This explains the higher expression of αSMA in the myocardium of CHD patients without cardiomyopathy compared with ICMP patients (Table 2). The αSMA protein is synthesized by vascular smooth muscle cells, which are the most numerous in the vascular wall, providing sustenance of vascular tone [13, 28]. Considering that the specific vascular area in CHD patients both without and with ICMP was comparable, and αSMA expression was lower in patients with ICMP (Table 2), therefore, it can be concluded that vascular volume is not altered in ICMP, but the structure of the vascular wall is obviously disturbed. Specifically, in ICMP, newly formed vessels are immature, and existing vessels are likely to lose tone, exacerbating ischaemia and causing myocardial contractile dysfunction and progression of heart failure.

Ang-2 is a negative regulator of angiogenesis since it blocks the binding of proangiogenic Ang-1 to their common receptor Tie-2, destabilises early vessels, and increases their permeability [29]. Ang-2 in conditions of VEGF-A excess, however, can be a Tie-2 agonist and activate angiogenesis, and in the absence of VEGF-A excess, Ang-2 accumulation is associated with vascular regression [23]. Therefore, increased

Ang-2 concentration in sinus blood in patients with ICMP compared with CHD patients without cardiomyopathy, while the level of VEGF-A in coronary blood flow was comparable between them (Table 2), can be considered as a sign of impaired angiogenesis in ICMP. Ang-2 and MMP-9 are considered as markers of cardiovascular disease, atherosclerosis and endothelial dysfunction [6]. MMP-9 degrades extracellular matrix components, including fibronectin [24, 30], which is part of the basolateral membrane of blood vessels [31]. This can either promote angiogenesis or vascular damage [6, 30]. Considering that in ICMP patients the content of MMP-9 and DEC in sinus blood was higher than in peripheral blood, while in CHD patients without cardiomyopathy it was the same (Tables 1, 2), the hypersecretion of MMP-9 in myocardium probably indicates its angiodestructive effect.

The results of the study may be limited by the clinical status of patients, provided that the data obtained are valid for CHD patients with haemodynamically significant multivessel lesions of the main coronary arteries. Consequently, these patterns may not yet be evident in patients in the early stages of ICMP formation, which requires further studies. The results were obtained for individuals of Caucasoid origin living predominantly in the Siberian Federal District.

CONCLUSION

To date, studies of ICMP mechanisms consider the imbalance of different types of collagen, cardiomyocyte apoptosis, impaired Ca^{2+} homeostasis and myocardial contractile function, vasomotor dysfunction of microvessels as its pathogenetic factors. However, the mechanisms of angiogenesis in ICMP patients have not been studied before. The present study has revealed that in CHD, complicated and uncomplicated by ICMP, two different variants of its pathogenesis are realized: with and without impaired angiogenesis. The progression of CHD without cardiomyopathy is accompanied by a compensatory increase in the mobilization of EPCs from the bone marrow in response to atherogenesis by excess SDF-1 in the blood. EPCs are actively recruited to the heart by VEGF-A and PDGF. Mature vessels containing sufficient smooth muscle cells (expressing αSMA) are formed in the myocardium as a result of PDGF secretion; therefore, activation of angiogenesis limits the progression of ischaemia and endothelial desquamation remains moderate. ICMP formation is associated with the absence of increased EPC mobilization, which are attracted to the myocardium by the action of VEGF-A alone, where, without the involvement of PDGF, immature vessels are formed that are easily degraded by Ang-2 and MMP-9. Such angiogenesis is obviously inadequate to the degree of vascular damage and forms a vicious circle of myocardial ischaemia in ICMP. The obtained knowledge about the mechanisms of dysregulation of angiogenesis in ICMP defines targets for its angiogenic therapy, the development of which will enable to slow down the progression of this severe disease.

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Conflict of interest

The authors of this article declare no conflicts of interest.

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Information about the authors

Svetlana P. Chumakova – Dr. Sc. (Med.), Docent, Professor at the Department of Pathophysiology, Research Officer at the Central Research Laboratory, Siberian State Medical University, e-mail: chumakova_s@mail.ru, https://orcid.org/0000-0003-3468-6154

Olga I. Urazova – Dr. Sc. (Med.), Professor, Corresponding Member of the RAS, Head of the Department of Pathophysiology, Researcher at the Central Research Laboratory, Siberian State Medical University, e-mail: urazova72@yandex.ru, https://orcid.org/0000-0002-9457-8879

Vladimir M. Shipulin – Dr. Sc. (Med.), Professor, Honored Scientist of the Russian Federation, Chief Research Officer, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: shipulin@cardio-tomsk.ru, https://orcid.org/0000-0003-1956-0692

Irina V. Sukhodolo – Dr. Sc. (Med.), Professor, Professor at the Department of Morphology and General Pathology, Siberian State Medical University, e-mail: suhodolo.iv@ssmu.ru, https://orcid.org/0000-0001-9848-2068

Angelina I. Stelmashenko – Teaching Assistant at the Department of Morphology and General Pathology, Siberian State Medical University, e-mail: staranie@mail.ru, https://orcid.org/0000-0002-8284-6864

Olga A. Denisenko – External Doctorate Student at the Department of Pathophysiology, Siberian State Medical University, e-mail: olga-muraveinik@yandex.ru, https://orcid.org/0000-0003-4524-8491

Sergey L. Andreev – Cand. Sc. (Med.), Research Officer, Cardiovascular Surgeon at the Cardiosurgical Department No. 1, Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: anselen@rambler.ru, https://orcid.org/0000-0003-4049-8715
Maksim S. Demin – Postgraduate at the Department of Pathophysiology, Siberian State Medical University, e-mail: maksimd25111996@mail.ru, https://orcid.org/0009-0001-0951-3158
Elena G. Churina – Dr. Sc. (Med.), Professor at the Department of Pathophysiology, Siberian State Medical University; Professor at the Department of Organic Chemistry, National Research Tomsk State University, e-mail: Lena1236@yandex.ru, https://orcid.org/0000-0002-8509-9921